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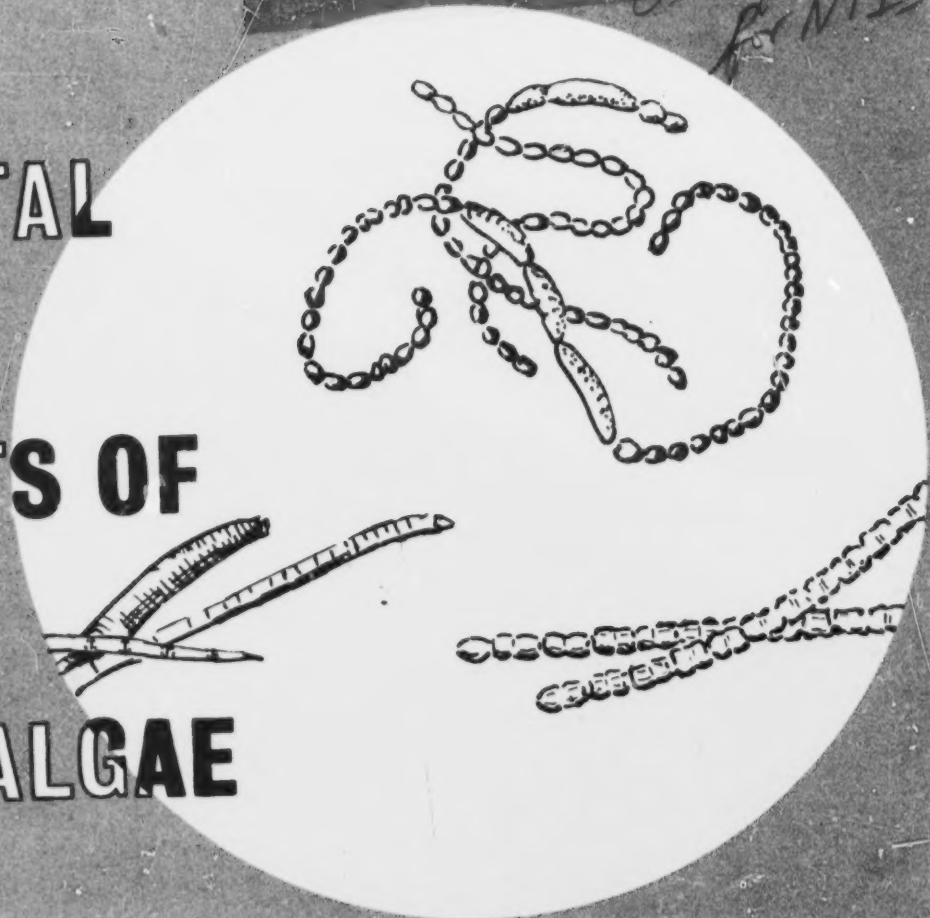
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ENVIRONMENTAL

REQUIREMENTS OF

BLUE-GREEN ALGAE



PROCEEDINGS OF A SYMPOSIUM
September 23-24, 1966

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16. Abstract The problem of accelerated eutrophication has many facets, and some are being examined through research to find the keys for solution. This symposium was held to fulfill the need to understand better the environmental requirements of blue-green algae. It contains eight papers by scientists who are concerned with the ecological control of algae blooms. Current knowledge on the subject has been evaluated and gaps identified for future investigation. The papers are followed by a general discussion of nutrient measurement and nuisance control. (See also W69-03513 thru W69-03518)				
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OF BLUE-GREEN ALGAE

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U. S. DEPARTMENT OF THE INTERIOR
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Corvallis, Oregon

October 1967

FOREWORD

The problem of accelerated eutrophication is of growing public concern. It is scrutinized increasingly not only by the scientist but by the public and its representatives in Government. There are many facets to the problem, and some are being examined through research to find the keys for solution. One of the most challenging areas is the need to understand better the environmental requirements of blue-green algae. Without this understanding it is doubtful the tools of ecological control now available can give relief from the ravages of such algal blooms. This subject area, therefore, cries out for research attention. It is for this reason that this symposium was arranged so that the knowledge now available can be evaluated, the gaps identified, and the researcher stimulated to pursue his inquiry in the light of this knowledge.

A. F. BARTSCH, Chief
National Eutrophication
Research Program

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WHY STUDY BLUE-GREEN ALGAE?

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The purpose of this introductory note is to give a brief review of some of the problems associated with blue-green algae that make it possible and useful to organize a symposium such as this.

One of the problems of current public interest is that many of the species of algae that cause the most difficult nuisance problems in lakes are blue-green algae. This is not to say that most species of blue-green algae are nuisances; many are inconspicuous and one never hears about them except in specialized literature. Nor does it mean that other kinds of algae do not cause problems; diatoms and Synura are well known to water filtering plant operators, but I do mean that any of a certain group of species can become very prominent in lakes, given the right environmental conditions, and there create the familiar nuisance conditions of scum and odor so well known in many parts of the world.

This feature is especially impressive to people who have seen an unproductive lake with an inconspicuous blue-green alga component in the phytoplankton turn into a productive lake with a relatively dense blue-green population. Lake Washington in 1950 was in relatively good condition in regard to algal population. It had at the maximum $1.5 \times 10^6 \mu^3/\text{ml}$ of total phytoplankton volume of which 15% consisted of species of blue-green algae. In 1962 the corresponding figures were 10.5 and 95%. By this time the lake had given rise to such public concern that construction of an elaborate and expensive sewage diversion program was underway (Edmondson, 1968; Clark, 1967).

But even beyond the special feature of creating public nuisance conditions, the blue-green algae would attract investigation by scientists because of their special properties. Anyone interested in working out the mechanisms of control of productivity and population density in aquatic communities would have to give special attention to these algae because they have special nutritional and other properties. Also, the blue-greens are conspicuous in a number of special or extreme kinds of habitats, such as hot springs. They are very important in forming and stabilizing soil. And, taking a broader view beyond the scope of this symposium, the specialized morphology and life history provide

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much additional motivation for study. Thus, there are scientific reasons as well as practical ones for working on these organisms and for holding a review symposium about progress.

Which special features of the blue-greens are involved? One is that the relation to gravity of some planktonic species is upside down. In calm weather, most of the nonflagellated algae settle toward the bottom of the lake as the diatoms do. In contrast, many of the blue-green algae float under these conditions and accumulate as dense scums on the surface which can be thrown into thick windrows and piled up on the shore. It seems probable that much of the material produced by the blue-green algae decomposes in the epilimnion rather than being deposited into the hypolimnion. This can have very interesting repercussions in the way the consumers in the different parts of the lake are supported.

We are concerned here with environmental conditions. Nutritional conditions are of considerable importance in the control of algal productivity and will be taken up first, but there is much more to the environment than the dissolved nutrients, and to interpret the effect of the supply of nutrients in nature, one must pay considerable attention to other processes. A real evaluation of the control of phytoplankton populations must recognize the quantitative importance of grazing, parasitism, antibiosis, and the difference in the light and temperature climate at different depths in the lake.

The blue-green algae for a long time have had a reputation for needing an organic source of nitrogen or at least for doing best under these conditions. This reputation apparently developed because the lakes in which the most prominent populations of blue-green algae occurred were lakes that had the highest concentration of organically combined nitrogen (e.g. Pearsall, 1934). On the other hand, it has subsequently been shown that the major nuisance species can be grown in pure mineral media without organic additives (e.g. Gerloff, Fitzgerald, and Skoog, 1952).

Is there an alternative explanation to this apparent paradox? A possible hypothesis is that while organic molecules are not obligatory, some organic compounds might be acting as a nutrient-sparing factor. Another reasonable hypothesis can be established on the basis that the blue-green algae on a relative basis need higher concentrations of inorganic nutrients for a given amount of growth than their normal competitors, such as diatoms and green algae. Now it happens that the lakes in Pearsall's group that had the highest concentrations of nutrients were the lakes that were enriched with sewage. Naturally, these lakes also had the highest concentrations of

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organically combined nitrogen. This coincidence was interpreted as a causal sequence by Pearsall.

It is still a good question which, as far as I know, has not yet been fully investigated, to what extent blue-green algae may be affected by organic substances even though they do not require them. The lesson to be learned from Rodhe's phosphate sparing factor should not be forgotten. Rather, this phenomenon should be actively looked for with other kinds of algae (Rodhe, 1948).

If it were true that the bloom species of blue-green algae need higher concentrations of nutrients to support a given rate of growth than do diatoms, one must ask why. It seems reasonable that this should be so because the bloom species form colonies. The cells in the middle of a colony of Microcystis are separated from the medium in which nutrients are dissolved. To support these cells with a given rate of nutrient absorption and growth equivalent to the same cells free in the medium, nutrients would have to be supplied to the colony at a higher concentration. The same argument could be applied to filamentous forms like Oscillatoria in which significant fraction of a cell surface is out of contact with the medium. If the colonial blue-green algae are put at a disadvantage by the colonial habit so widespread in these forms, one wonders how coloniality could have evolved. A possible answer in this seems to be that the colonial species may be less readily eaten by some zooplankton, particularly Daphnia, Diaphanosoma, and Diaptomus. Experimental data support this idea (Sorokin, et al., 1965). Thus, the colonial habit represents an antipredator device of some effectiveness. Of course, it must be emphasized that it is a relative protection. When a Diaphanosoma is seen to wad up a bunch of Oscillatoria and discard it, it seems obvious that the Oscillatoria has not been eaten. Nevertheless, small fragments may be broken off in this process and contribute to the nutrition of the animal.

To make a complete accounting we must consider in some detail what the predatory loss to the zooplankton is, and generalizations at the moment do not seem obvious. For example, Hrbacek has found fish-ponds near Prague which support a dense population of Aphanizomenon and also Daphnia in good conditions as judged by appearance and presence of eggs (personal communication). It is not at all clear what is going on but it has been supposed that a large proportion of the material photosynthesized by blue-green algae is leached out into the environment and supports a production of bacteria, which in turn supports Daphnia. One can also suggest that the feeding mechanism and behavior of the various species of Daphnia need comparative examination.

One may question the proposition that blue-green algae need high concentrations of nutrients because large populations are found with

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low concentrations of nutrients. For instance, in 1962 a dense population, dominated by *Oscillatoria agardhii*, was maintained at a high concentration in Lake Washington for several months with concentrations of P_2O_4 less than $10 \mu\text{gm/l}$ and $N.NO_3$ less than 50. But during the period of rapid growth in April and May, the concentrations were more than 30 and 300, respectively.

Thus, the proper evaluation of field data is critical here. It seems incorrect to describe as optimal the nutrient conditions at the time a population is not able to grow or even about to crash.

The matter is probably not quite so simple as implied in the foregoing discussion which put emphasis on the response of growth to nutrient concentration. Provasoli (1958) has suggested that the proportions of the major cations may be important in determining which particular species shall predominate in enriched lakes. Lakes which receive considerable sewage are relatively enriched in sodium and chloride. Since some species of Myxophyceae have a requirement for sodium or potassium, there is opportunity for a selection of species to be made on a basis other than competition for nitrate, phosphate, or other exhaustible nutrients. Lakes enriched primarily with agricultural fertilizers should have ionic ratios somewhat different from those enriched primarily with sewage, and a close examination of the situation in such lakes should be instructive.

At present it seems not possible to clarify this question with existing data. We need to have more specific quantitative information about how the rate of cell growth and division varies with nutrient concentration. A considerable body of experimental literature has accumulated that shows the maximum amount of algal material develops in media of different nutrient concentration (e.g. Gerloff and Skoog, 1957), but few reports show how the growth rate varied during the period of growth during which the accumulation took place.

The effect of selection or differential filtration by planktonic and benthic animals needs further study to establish or disprove the mechanism hypothesized here. While it has been shown qualitatively in a general way that the larger colonial species of blue-green algae are not consumed as readily as the unicellular greens, we seem not to be in a position to make meaningful calculations of rates of production and consumption of the different algal types. While this degree of detail may not be required to settle this problem, it would be desirable to have that much knowledge.

What other mechanisms are there of population control other than nutrient limitation and grazing? There has been a great deal of discussion of antibiotic substances liberated by algae. While literature

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is to some extent ambiguous and contradictory, it appears from many works that algal antibiosis is a real phenomenon in nature (e.g. Vance, 1965). Again, we have a qualitative evaluation and need to know much more quantitatively about such matters as the degree to which cell division is slowed by under specified conditions. Parasitism is another process for which quantitative evaluation is desirable.

To conclude I should like to mention the Oscillatoria rubescens problem.

It has been said that in 1955 Lake Washington was obviously on its way to deterioration. What was really meant was that suddenly a large population of Oscillatoria rubescens appeared. This species has frequently shown up early in the deterioration of lakes brought about by sewage enrichment. Of course, it is not restricted in its occurrence to sewage-enriched lakes, but there is something about these conditions that makes it do well and form dense populations. This species has been present in Lake Washington since 1955, but not uniformly. It has come and gone, being succeeded or accompanied at times by other species, including Oscillatoria agardhii.

It seems a worthwhile task to define the conditions in which Oscillatoria rubescens does well. Staub (1961) published a very thick paper on the problem which he described as a first small step forward. And, in fact, it is difficult to state on the basis of this why Oscillatoria rubescens occurs where and when it does.

So there are still bits and pieces that need to be fitted together. I hope no one will take offense at the description of the very large literature and hard work that has been done on these organisms as bits and pieces. All this means is that there are some very interesting ecological problems, centered on environmental requirements, that still need to be investigated.

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PROBLEMS IN THE LABORATORY CULTURE
OF PLANKTONIC BLUE-GREEN ALGAE

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Both major orders of blue-green algae, the Chroococcales and the Hormogonales, are represented in the common plankton communities of the fresh water. Furthermore, forms may be either occasional or rare members of the community, or they may be dominant, i.e., comprising the vast majority of a given sampling, often reaching bloom proportions. Still another approach to plankton algae divides species into epilimnetic forms (warm temperature, bright light) and hypolimnetic or metalimnetic forms (cool temperature, dim light). Generally speaking, epilimnetic blooms may be dominated by either members of the Chroococcales (Microcystis) or by some filamentous forms (Anabaena or Aphanizomenon), while deep-water blooms are nearly always composed almost exclusively of one of several species of Oscillatoria.

With one exception, the writer has been concentrating on the filamentous planktonic forms, with special reference to the Oscillatorias associated with deep-water oxygen maxima. In the United States, metalimnetic oxygen maxima may be produced by several types of phytoplankton communities, one of which is designated the "Oscillatoria type" (Eberly, 1964b). In two lakes in Indiana which have been extensively investigated, the alga has been identified as Oscillatoria agardhii (Eberly, 1959, 1964a). This alga produces intense mid-summer blooms at depths where the temperature is 10-12°C and the light intensity usually less than 1% of the surface intensity. Under these conditions, and with a stability imposed by the thermal gradient and various morphometric characteristics, oxygen saturations exceeding 30 ppm have developed on occasion.

High oxygen concentrations in lakes of central Europe are associated with Oscillatoria rubescens (Eberly, 1964b). Most of these lakes also reflect an increasing rate of eutrophication due to nutrient additives present in treated sewage effluent. In fact, O. rubescens is often taken as an indicator of pollution or eutrophication. In Lake Washington, O. rubescens has succeeded O. agardhii as eutrophication has advanced over the years (Edmondson, 1961; Anderson, 1961). Preliminary evidence seems to suggest that a similar transition is taking place in McLish Lake (Indiana), though this has not been determined with any certainty yet. This, then, provides a second reason for studying these and related species.

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Still a third reason for studying this group of organisms is related to their taxonomy. There are a number of planktonic Oscillatorias with non-constricted cell walls and prominent pseudovacuoles which are very nearly indistinguishable. Preserved specimens differ chiefly in diameter of the cells. Living cultures show delicate differences in shading, ranging from olive green to deep red, with all shades in between. Various species grown in different media and under different light and temperature conditions will exhibit different colors and shades. Hopefully, some progress might be forthcoming in the area of the systematics of this very similar cluster of species.

Obviously, the objectives or purposes of an investigation affect the design and conduct of the experiment. Various studies of blue-green algae purport to study processes or functions of the organism (photosynthesis, mineral nutrition, etc.), while other studies intend to relate the organism to the environmental conditions under which it lives in nature. Most experiments will contribute some degree of understanding to both aspects of the problem, and, indeed, a fine line between the two types of experiments is difficult to draw. Nevertheless, the difference does exist. My own work has been largely ecologically and taxonomically oriented. Consequently, the pure physiologist or biochemist may find much to be desired in the data presented here. It should indicate, however, areas where further work may prove to be of value. Cultures of most of the species and strains described in this paper may be obtained from the author.

Some of the work reported here was done while the writer was participating as a visiting scientist at the Institute of Limnology of the University of Uppsala in Sweden during the academic year 1963-64 (Eberly, 1965). Seven species were studied while at Uppsala, of which three were described as new species or new varieties (Eberly, 1966). A number of these cultures have been maintained at the Institute of Limnology by Dr. Wilhelm Rodhe and his co-workers and were forwarded to North Manchester after facilities were developed there. Five cultures were sent the author by Dr. Olav Skulberg of the Norsk Institutt for Vannforskning at Blindern, Norway. A word of thanks is due both Dr. Rodhe and Dr. Skulberg and their respective institutions for their assistance in this study. The writer is also deeply appreciative of the continued and generous support of the National Science Foundation. Thanks, also, to Misses Bonnie Kirby and Jane Henney, who served as research assistants during much of this study, and to Miss Cec Widup, who typed the manuscript.

METHODS

All of the work reported here has been done with unialgal cultures, none of which is bacteria-free. Single filaments were easily isolated by plating several drops of culture on a 1.5% agar mixture of standard medium. Most species of Oscillatoria soon crawled away from the site of the original inoculum and could be picked up with a fine needle and placed in test tubes. Single filaments of blue-green algae grow very slowly in this kind of isolation. Many do not survive. This may be related to a dependency upon some diffusable metabolic factor, as Fogg (1965) suggests in connection with the development of the lag phase of the growth curve, or it may be a purely physical factor related to the buoyancy of the filament, which causes it to creep up the water film along the side of the test tube and, subsequently, to dry out with the evaporation of the medium. Needless to say, regardless of the number of isolates prepared, successful growth of only one is necessary to provide the desired culture. Experimental cultures were maintained in 125 ml Erlenmeyer flasks stoppered with a plug of medical quality cotton. Inoculations were made into about 50 ml culture medium. The flasks were shaken continuously at about 100 cycles per minute. Illumination was provided by 40-watt Sylvania F-40 natural tubes, operating on a 12-hour, on-off cycle. Intensity at the level of the flasks was about 70-85 ft.-c. Temperature in the two growth chambers was maintained at 15° and 20°C. Growth of the cultures was measured turbidometrically, using a Fisher Model 7-101 electrophotometer with a color filter with a peak transmission at 650 mμ. The optical density of the measurements is multiplied by 100 to eliminate the decimal. No attempt was made to relate optical density to absolute quantity of algae present, though this has been done by other authors. For example, Staub (1961) measured growth of cultures of Oscillatoria rubescens with a filter of 610 mμ and prepared a graph giving the dry weight of algae as mg/l plotted against the extinction value. This gave a straight line, so it was felt that a similar condition would exist with the algae used in this investigation (though with likely different absolute values).

Cultures were grown both in the ASM and ASM-1 media of Gorham et al. (1964). Table 1 gives the composition of these media in micromoles of ingredients. In almost every case, growth was faster and reached higher population densities in ASM-1 than in ASM. Specific results will be presented later in connection with the discussion of each species. No further attempt was made to define a specific formula for each culture used. No doubt, some differences might be discovered, but the filamentous forms do show quite successful growth in the one medium.

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Cultures Currently Under Investigation

At the present time, 19 species and strains are under investigation in the writer's laboratory. Nine of these were isolated from the 8-meter-deep sample from McLish Lake (Indiana) obtained on August 3, 1965. These comprise two species, Oscillatoria agardhii and Oscillatoria limnetica. Their culture numbers bear the prefix E (Eberly). Five cultures are forms isolated from Swedish waters and bear the prefix UP (Uppsala). A fuller description of these forms is found in Eberly (1966). Five cultures were sent to the writer by Dr. Olav Skulberg from Norway and are designated by the prefix Sk. Four of these were isolated from Norwegian lakes, and the fifth is from the clonal culture of O. rubescens isolated by Richard Staub from Lake Zurich in Switzerland (Staub, 1961). The nomenclature is presented here in the traditional sense. The systematics of these forms is under study and will be discussed elsewhere.

- E7. Oscillatoria agardhii. McLish Lake. Brown.
- E8. Oscillatoria agardhii. McLish Lake. Reddish brown.
4.5-5.0 μ thick.
- E10. Oscillatoria agardhii. McLish Lake. Gray brown.
4.5-4.7 μ thick.
- E12. Oscillatoria agardhii. McLish Lake. Brown. ca. 6.0 μ
thick.
- E18. Oscillatoria agardhii. McLish Lake. Olive brown.
ca. 6.0 μ thick.
- E19. Oscillatoria agardhii. McLish Lake. Olive brown.
ca. 6.0 μ thick.
- E16. Oscillatoria limnetica. McLish Lake. Deep blue-green.
- E20. Oscillatoria limnetica. McLish Lake. Green blue-green.
- E40. Oscillatoria limnetica. McLish Lake. Bright blue-green.
- Up1. Oscillatoria agardhii isothrix. Lake Hangstaorn, Sweden.
Green blue-green.
- Up2. Oscillatoria baltica. Baltic sea off Stockholm, Sweden.
Green blue-green.
- Up3. Synechocystis parvula. Baltic waters near Stockholm.
- Up4. Oscillatoria limnetica. Lake Norrviken, Sweden. Blue-
green.
- Up6. Oscillatoria baltica. Lake Norrviken.
- SkA. Oscillatoria agardhii. Lake Gjersjo, Norway. Green blue-
green.
- SkB. Oscillatoria agardhii isothrix. Lake Froilandsvatn,
Norway. Green blue-green.
- SkC. Oscillatoria rubescens. Lake Zurich, Switzerland.
(Staub). Red brown.

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- SkD. Oscillatoria rubescens. Lake Steinsfjord, Norway. Light red-brown.
SkE. Oscillatoria agardhii. Lake Akersvatn, Norway. Deep blue-green.

EXPERIMENTAL RESULTS

Oscillatoria agardhii Gom.

Most of the work on O. agardhii was done with strain E10 from McLish Lake in northern Indiana. In ASM medium, a rather characteristic growth curve is exhibited. The stationary phase was generally reached at an optical density of about 45 and lasted about 15 days before declining in the death phase (the terminology is that of Fogg 1965). The so-called "lag phase" did not seem to be a static period, but, rather, indicated a steady but small amount of growth from the beginning. The duration of the lag phase varied rather directly with the concentration of the culture at the beginning of the experimental period, i.e., the higher the concentration of algae at the beginning, the shorter the lag phase (Figure 1). With an initial optical density of 5 or greater, a lag phase was scarcely apparent at all. No attempt was made to ascertain the physiological state of cells of the inoculum, but, in general, the stock cultures were maintained within the exponential phase of growth.

Brief work with the pH of the culture solutions indicated that, regardless of the initial pH of the medium, within a matter of a few days a rather uniform pH is attained. Adjustment of pH was accomplished by adding HCl or NaOH. Cultures started at pH 7 to 10, dropped to 6.6 to 7.4 by the second day, while cultures at pH 6 or below rose to about 6.5 to 6.8. No attempt was made to adjust the pH at daily intervals as has been done by other workers. In cultures with initially varying pH values, the exponential phase of growth was reached quickest in the cultures with the higher pH, but the level at which the stationary phase developed was generally similar in all cases and persisted for similar periods of time (Figure 2). The curve for an initial pH of 9 is close to that of pH 8 and 10, while the curve for pH 5 is similar to that of pH 6. The pH in each culture at the time of the beginning of the exponential phase was about 7.1 to 7.3. At the height of the exponential period, the pH rose to 9 to 9.2 for each culture, with the highest maximum pH values observed being in those cultures with initial pH values both higher and lower than pH 7. During the stationary period, all pH values declined to 7.9 to 8.1. As a standard procedure for subsequent experimental work, the pH of the medium was adjusted to 7.5-8.0 before inoculating with algae.

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In a simple experiment to see if any unknown substance had any effect on growth, a culture of *O. agardhii* was augmented by the addition of 1% and 10% soil extract prepared from ordinary loam silt soil according to the directions of McLachlan et al. (1963). The results (Figure 3) indicate that the soil extract stimulated growth earlier than the plain ASM and produced maximum growth at a somewhat higher concentration, but the duration of the stationary phase was roughly the same for all three cultures. A comparison of the three curves indicates that the differences are quantitative rather than qualitative. This suggests that there is no mysterious growth substance involved, but simply an increased concentration of a vital inorganic nutrient in the soil extract. It is, of course, impossible to standardize any kind of soil extract so that comparisons can be made with different solutions.

It was noted early that somewhat more rapid growth was achieved in ASM-1 medium, as well as greater concentrations during the stationary period nearly twice that of the ASM cultures (Figure 5). In ASM-1, strain E10 leveled off at an average optical density of about 80. In order to determine which ingredient of the ASM-1 medium was responsible for the increased growth, replicate cultures of strain E10 were started in ASM medium and cultured under conditions described earlier until about the middle of the stationary phase. Then each nutrient that was present in ASM-1 in a different concentration than in ASM was added to separate cultures in the amount representing the actual difference between the amount of that nutrient in 50 ml of ASM-1 medium and 50 ml of ASM. These additions were made on the 18th day. Only the addition of NaNO_3 produced increased growth which approximated the level achieved with ASM-1 medium (Figure 4). It is clear from this that nitrate is the limiting factor in the ASM medium under these growth conditions. Whether *O. agardhii* can obtain nitrogen from any other substances is not known. This experiment may also cast some doubt on the ability of this alga to utilize free nitrogen.

The growth curves representing growth after the addition of aliquot amounts of FeCl_2 , H_3BO_3 , and CaCl_2 are almost identical with the growth in untreated ASM medium. The addition of Na_2HPO_4 depressed growth. This appears not to be due to the addition of sodium, since additional sodium was added in the culture with the NaNO_3 supplement. Perhaps the increased amount of phosphate could not be tolerated in the presence of a depleted supply of nitrate. The most striking effect came with the addition of the cobalt-copper-zinc salts (which were added in a common solution). The algae were killed and began to lyse very quickly. Since the three ions were added together, it is not known whether this is a result of one or several. This provides an

excellent illustration of the fact that the toxicity of many heavy metals is determined in part by the concentration of other substances present in the water.

The two Norwegian strains of O. agardhii (SkA and SkE) also responded best in the ASM-1 medium (see Figures 6 and 7). Unfortunately, the maximum growth exceeded the capacity of the instrument to measure it. It should be remembered, that an O.D. of 100 represents a maximum absorbance of 90%, so the actual maximum yield of any alga giving an O.D. over 100 could not be over 10 to 15% greater than the maximum yield of O. agardhii (strain E10) in ASM-1.

Figures 5-13 present the results of an experiment in which nine different cultures were grown in both ASM and ASM-1 media at both 20° and 15°C. Initial densities were similar in all cases. The particular pattern of the four growth curves for each culture gives something of a systematic characterization of that form in comparison with the others. Suffice it to say at this point that SkA and SkE appear very similar to each other and that they both give a somewhat different picture than strain E10. How this relates to the taxonomy of the species remains to be seen.

The general effect of the lower temperature is merely to retard the rate of growth. In an earlier experiment with strain E12, cultures at 15°C reached nearly the same maximum density as cultures at 20°C, but about two weeks later. In Figures 6 and 7, the retardation of the colder cultures is apparent, but it is not possible to determine the maximum yield of these cultures yet. It is clear that these two strains grow as fast and produce higher yields in ASM-1 at 15°C as they do in ASM at 20°C. The growth of strain E10 was more rapid in ASM-1 at 15°C than in ASM at 20°C. This points up the fact that often the growth response of an alga to a particular combination of light intensity and temperature is determined in part by the ionic concentration of the environment.

Oscillatoria agardhii var. isothrix Skuja

Of the two strains of this variety, only one (SkB) has been subject to experimentation so far in our laboratory. Some work was done on strain Up1 at Uppsala and will be referred to later. The growth response pattern of SkB (Figure 8) is similar to that of SkA and SkE. If isothrix is a valid subspecies of O. agardhii, one would not expect to find drastic differences in physiological adaptation. The slight difference in growth of SkB in ASM at 20°C and ASM-1 at 15°C may or may not be significant. The extremely slow growth in ASM at 15°C might also be

ENVIRONMENTAL REQUIREMENTS OF BLUE-GREEN ALGAE

pointed out as a minor deviation of this form. This particular growth curve nearly parallels that of strain E10, though the two cultures differ in other respects.

Oscillatoria baltica Eberly

One strain of this species (Up2) was used in experimental work. The growth response pattern (Figure 9) bears a striking resemblance to that of strain E10. The maximum concentration was not so great as in E10, and the stationary phase was very short preceding the rather abrupt decline of the culture. There is a consistent difference between growth in ASM-1 at 15°C and ASM at 20°C, with the former being decidedly greater. The relatively minor effect of temperature in the case of the two ASM cultures might also be pointed out.

Oscillatoria rubescens D.C.

The difference in the response pattern of the two cultures of O. rubescens SkC and SkD may be noted in Figures 10 and 11. Though both strains grew best in ASM-1, SkC reached a peak density of about 65 and maintained a prolonged stationary phase. On the other hand, SkD peaked at an optical density of about 86 and began to decline rather quickly. Strain SkC produced a pair of growth curves in ASM similar to those of ASM-1, showing rather simple quantitative differences produced by the differing temperature and ionic concentrations of the two media. Strain SkD, however, did not grow at all in medium ASM. In the light of the profuse growth in ASM-1 at 20°C, the results in ASM are difficult to explain. It should be recalled that SkC is originally from Lake Zurich in Switzerland, while SkD is from Norway. The identifications are by the original isolators of the cultures.

Because of its association with polluted and eutrophic waters, an experiment was set up to determine the effect of the addition of treated sewage effluent to cultures of O. rubescens in ASM medium at 20°C. The results are shown in Figure 13. The sewage supplement produced a more rapid growth, but the maximum yield was lower than in plain ASM medium. These results were hardly what had been expected and did not illuminate the problem very much. The particular sample of sewage effluent, obtained from the outlet of the disposal plant at Wabash, Indiana, was not analyzed chemically.

Laboratory Culture of Planktonic Blue-Green Algae

Oscillatoria limnetica Lemm.

The growth pattern of this form (E40) is shown in Figure 12. The rate of growth of cultures at 15°C compared with the growth in the same medium at 20°C was more nearly the same than in any of the other cultures. The quantitative relationship of maximum growth indicates rather clearly that the final yield (during the stationary phase) in ASM-1 is about double that in ASM. This is perhaps true of some of the others but shows most clearly in this experiment with O. limnetica.

Synechocystis parvula Perfiljev

The origin and taxonomy of this culture were discussed earlier by the writer (Eberly, 1966). The role of this organism in natural planktonic communities is unknown. In culture it grows very rapidly in a variety of media. In the present experiment the growth rate during the early part of the exponential phase was identical in ASM and ASM-1, at both temperatures, though the density of the culture during the stationary period was considerably less in ASM (Figure 14). The final density in ASM-1 considerably exceeded the 90% absorbency which the instrument was capable of measuring. Though it may not contribute too much to our knowledge of planktonic forms, a study of this organism may reveal a number of interesting variations in blue-green algae physiology.

TAXONOMIC NOTES

Although a fuller discussion of the taxonomy of the forms included in this study is under preparation, a few comments might be made here. The role of biotic characteristics in systematics, as contrasted with or superimposed on morphologic characters, is very uncertain at the present time, with the notable exception of the bacteria. In the blue-green algae, two biotic characteristics have contributed to the present state of confusion in the taxonomy of the group. One is the way in which the actual morphology of the organisms (size and dimension, cell shapes, presence or absence of sheaths, gross form of colonies or mat formation, etc.) vary with differences in environmental conditions. By growing pure cultures of a single species, Schizothrix calcicola, in a great variety of culture media and under a variety of environmental conditions, Drouet (1963) was able to classify the final growth forms as 54 species in nine different genera now included in the commonly used manuals. Other workers are investigating the same phenomenon in various groups of blue-green algae.

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The second factor is the variability of physiological response observed under different conditions. This may, in part, be merely the physiological counterpart of the morphological variability noted above, or it may actually represent genetically determined responses, i.e., differences valid at the species level. The latter kind of differences might be expected to show up when a series of cultures are grown under identical conditions and in identical nutrient solutions. The growth response graphs representing the growth of each culture in two different media and at two different temperatures present this kind of data (see Figures 5-11). Other biotic characters include color of cultures (preferably during the exponential phase of growth) and certain other characters, such as length of filaments, degree of bending of filaments, presence or absence of clumping, sheath, etc. Observations of this latter sort have been made, for example, on Aphanizomenon (McLachlan et al., 1963) and Anabaena (Gorham et al., 1964). How much (and even whether) such observations should enter the taxonomic picture is a very current topic. Certainly such information ought not to be disregarded. In the present study, color of cultures and growth response patterns are considered along with typical morphological parameters.

Strains SkA and SkE identified as Oscillatoria agardhii from two lakes in Norway are almost certainly identical. The color and general morphology are very much alike and the growth patterns are very similar (Figures 6, 7). Strain SkB, O. agardhii isothrix, also from Norway, is similar enough to the previous two cultures (Figure 8) that it very well could be the same species but with minor differences (chiefly morphological) at the subspecies level. The color is a somewhat lighter green. The strain from Sweden (Up1) has almost the same color as SkB, though the former has not yet been studied experimentally in our laboratory at North Manchester. Oscillatoria baltica, Up2, has the same green blue-green color as the above forms but has a different growth response pattern (Figure 9). Its continuation as a discreet species so far seems justified on the basis of its morphology and physiological response.

On the other hand, the form from northern Indiana identified as Oscillatoria agardhii (E10 and the others) does not seem at all like the O. agardhii from Norway. The color is brownish, ranging in the various strains from reddish-brown to gray-brown. The growth response graph of E10 (Figure 5) is more like that of O. rubescens (Figures 10 and 11), especially SkD. Assuming that SkC is the valid O. rubescens, some question could be raised about SkD. The filaments of SkD are about 9-10 μ thick compared with 5-6 μ in SkC. However, the filaments of E10 are only 4.5-5 μ thick.

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We are certainly dealing with two groups of algae: the green ones, that might be called the agardhii group, and the brownish ones, the rubescens group. How O. prolifica fits into the picture is not known. In O. rubescens the degree of reddish or brownish tint seems to be greatly affected by the growth conditions, particularly nutrients available. This is also true of E10. In the experiment on E10 shown in Figure 4, the culture with the added nitrate became almost as red as O. rubescens, strain SkC. The confusion has been that when dried or preserved specimens of these alga are examined they sometimes appear alike, while in the living condition they are very much different. It appears likely that what we have been calling O. agardhii in our lakes in Indiana is not that. Perhaps, also, what has been reported to be a transition from "O. agardhii" to "O. rubescens" is not actually a change from one species to another, but a transformation of a dull variety (such as E10) to a bright red appearance of the same form, with changing nutrient conditions in the lake. These relationships remain so far largely as speculations, but they form the basis of some experimental work for the future.

SOME PROBLEMS REMAINING

Besides the problems in the area of taxonomy alluded to above, some other problems remain yet unsolved. One of these relates to the difficulty of maintaining the algae in a truly planktonic or suspended state. At the present time, this can only be accomplished by artificial means, such as shaking, rotation, or some other mechanical means of inducing turbulence, or by bubbling air through the culture vessel. The degree of turbulence thus required to maintain the alga in a suspended condition is certainly greater than the actual amount of turbulence existing in a natural situation. In fact, one might say that usual culture methods produce such drastic conditions that it is a wonder the algae survive at all. A means of assessing the effect of this artificially induced turbulence on the growth data thus obtained is needed. Fogg (1965) has discussed this problem very briefly. He cites work that indicates that "growth of Anabaena cylindrica was doubled when culture flasks were shaken at 90 instead of 65 oscillations per minute and entirely prevented at 140 oscillations per minute." Further, we ought to try to maintain cultures in suspension without any artificially induced turbulence in the culture.

A second problem concerns the role of bacteria in the nutrition of blue-green algae. This, of course, is not a problem unique to planktonic forms. Only a few workers have attempted axenic cultures of blue-green algae which possess slime sheaths in which (or on which) bacteria may feed. In some cases (again see Fogg, 1965) various algal

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cultures (not necessarily blue-green) grew poorly or not at all in axenic bacteria-free cultures. Whether any symbiotic dependency on bacteria exists in planktonic blue-green algae is not known yet. A related problem is the possible production of antibiotic substances by the alga. These might be anti-bacterial or anti-algal. In nature, the massive deep-water blooms of this type of Oscillatoria are remarkably homogeneous, containing very few forms other than the dominant one. Whether this is a purely physical result of shading and temperature or the result of heteroantagonism (Lefevre, 1964) remains to be seen.

Another problem concerns the duplication of the spectral quality of light as it exists in nature. The attenuation of certain wave lengths of light in water is well known. It is, however, difficult to determine the actual spectral distribution of subsurface light and equally difficult to duplicate that quality in the laboratory. If chromatic adaptation is a reality in blue-green algae (as the writer is inclined to feel), then the actual color of an alga in nature is a response to the spectral composition of the light. This color could obviously not be attained exactly in laboratory culture unless the light quality was the same as in nature. To what extent a different quality of light gives deviant results in growth experiments is not known. It is fairly easy to use bulbs of differing and known spectral emissions, but this still does not quite present a true picture of growth under natural conditions.

Related to this is the growth response of the alga to light intensity and temperature. Rodhe (1948) was one of the first to point out that the response of an alga to light intensity depends on the temperature and vice versa. That is, a particular light intensity may be sub-optimal at a certain temperature, optimal at another, and supraoptimal (induce light saturation) at still another temperature. In theory, it is reasonably simple to expose replicate cultures to various combinations of light and temperature, but in practice it is somewhat more difficult. In addition to providing cross-gradients of light intensity and temperature for a number of samples, some method of keeping the algae suspended must be devised and the entire apparatus must be constructed with a particular method of measuring growth in mind.

Recent attempts to deal with this problem with algae other than blue-greens include that of Maddux and Jones (1964) and Jitts et al. (1964). While at the Institute of Limnology in Uppsala, this matter was investigated briefly with some of the cultures of blue-greens available at that time. Experiments were carried out in the photo-thermostats in Dr. Rodhe's laboratory. Two units were used at temperatures of 12° and 20°C. Growth was measured by the relative uptake

of carbon-14, with the greatest amount of uptake in any one experiment arbitrarily taken as 100%. Results are in cpm of a filtered aliquot of the sample. Cultures were acclimatized in the photothermostat in the dark for 12 hours before the light cycle came on. Light exposure was six hours. Cultures were placed in 125 ml pyrex bottles which were placed in a circular holder which rotated at right angles to the light source in the top of the chamber. This meant that each bottle was exposed to a varying light intensity at different points during the rotation of the culture holder, but that during a complete rotation each bottle received a similar amount of illumination. The actual amount of illumination received (or the average intensity) was not measured, but maximum intensity immediately under the lamps was perhaps close to 1,000 ft.-c. This is considerably higher than that used to cultivate blue-greens under normal laboratory conditions. In order to obtain varying levels of intensity, bottles were wrapped in one, two, or three thicknesses of copper screen which reduced the intensity to 52%, 26%, and 13% of the unshielded bottles.

Even though this was a somewhat crude way of measuring this particular response pattern, the data obtained did indicate some remarkable things. Data for four species are presented in Figure 15. At 20°C, *Oscillatoria agardhii isothrix* (Up1) had not yet reached a level of light saturation in the full unshielded bottles, while at 12°C some degree of light saturation was reached at 26% of the full illumination. *Oscillatoria limnetica* (Up4) likewise did not show any inhibition at 100% intensity but did show saturation at 12°C under 52% illumination. *Synechocystis parvula* (Up3) is apparently a real stenothermal alga. At 20°C, optimum light intensity is reached at 26% illumination, while growth at 12°C in the higher level of illumination exceeds that at 20°C. A similar light saturation of NRC-48 (an unidentified algae provided by Dr. Paul Gorham of the National Research Council at Ottawa) occurred at 20°C and 52% illumination, after which the amount of growth at full illumination fell below that at 12°C. A graph for *Aphanizomenon flos-aquae* was nearly identical with that of *O. limnetica*. These data are only preliminary, but they do indicate the kind of results that would be worth obtaining under more precise conditions.

The work of Maddux and Jones (1964) demonstrated that the growth response of a diatom *Nitzschia* indicated variable light and temperature optima depending on the ionic concentration of phosphate and nitrate in the medium. At high concentrations of these two nutrients, both the light optimum and temperature optimum were higher than at a lower concentration of nutrients. If this is true of the planktonic blue-greens, this might shed some light on the occurrence of various forms at certain limited depths in lakes. Under low concentrations of nutrients (i.e., oligotrophic or mesotrophic waters) optimum growth conditions would be

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reached at depths with reasonably low temperatures and limited light penetration. With an increase of nutrients (eutrophication) light and temperature optima might be raised, with the result that the maximum concentration of algae (the bloom) would move closer to the surface. This might also explain the downward movement of a bloom during the summer with the gradual depletion of nutrients which lowers the light and temperature optima. Such a seasonal downward movement of the bloom of Oscillatoria has been observed in Myers and McLish Lakes.

The greatest difference between ASM and ASM-1 is concentration of certain nutrients. Some of the results presented in Figures 5-12 might be interpreted in the light of the above principle. It would not be possible to determine exactly the light and temperature optima without experiments such as have been referred to before. It would also seem that this phenomenon would be operative only within a lower range of concentration of nutrients; that is to say, above a certain level of concentration, increasing the nutrient level would not have any further effect on light and temperature optima. This hypothesis also needs to be tested. All in all, this area of investigation promises to be a fruitful field of inquiry into the ecological physiology of planktonic blue-green algae.

Table 1
Composition, in micromoles, of ASM and ASM-1 media

SALT	ASM	ASM-1
NaNO ₃	1000	2000
MgSO ₄	200	200
MgCl ₂	200	200
CaCl ₂	100	200
K ₂ HPO ₄	100	100
Na ₂ HPO ₄	-	100
FeCl ₃	2	4
H ₃ BO ₃	10	40
MnCl ₂	7	7
ZnCl ₂	0.8	3.2
CoCl ₂	0.02	0.08
CuCl ₂	0.0002	0.0008
Na ₂ EDTA	20	20

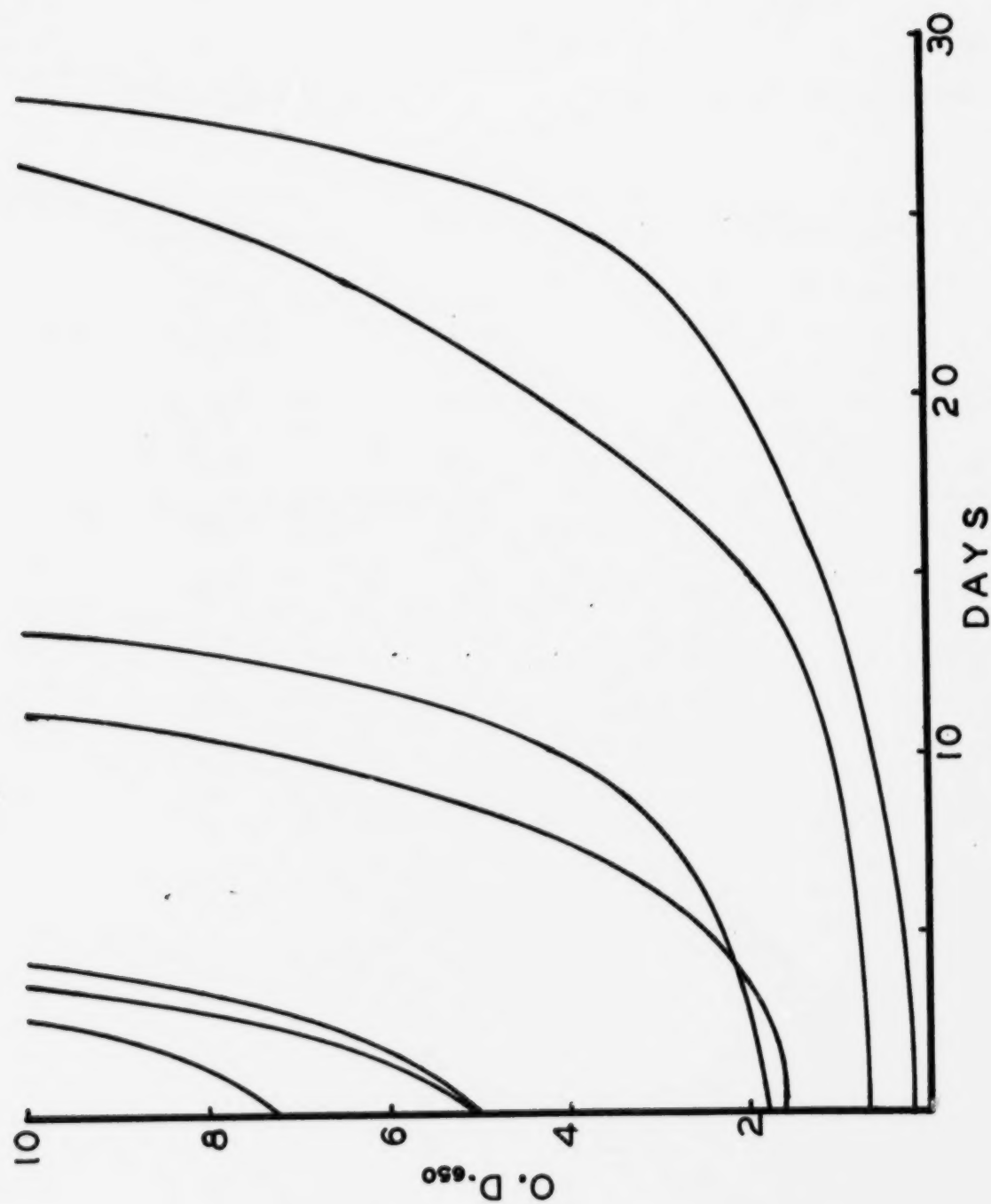


Figure 1. Effect of the initial concentration on the lag phase of the growth curve of *Oscillatoria agardhii*.

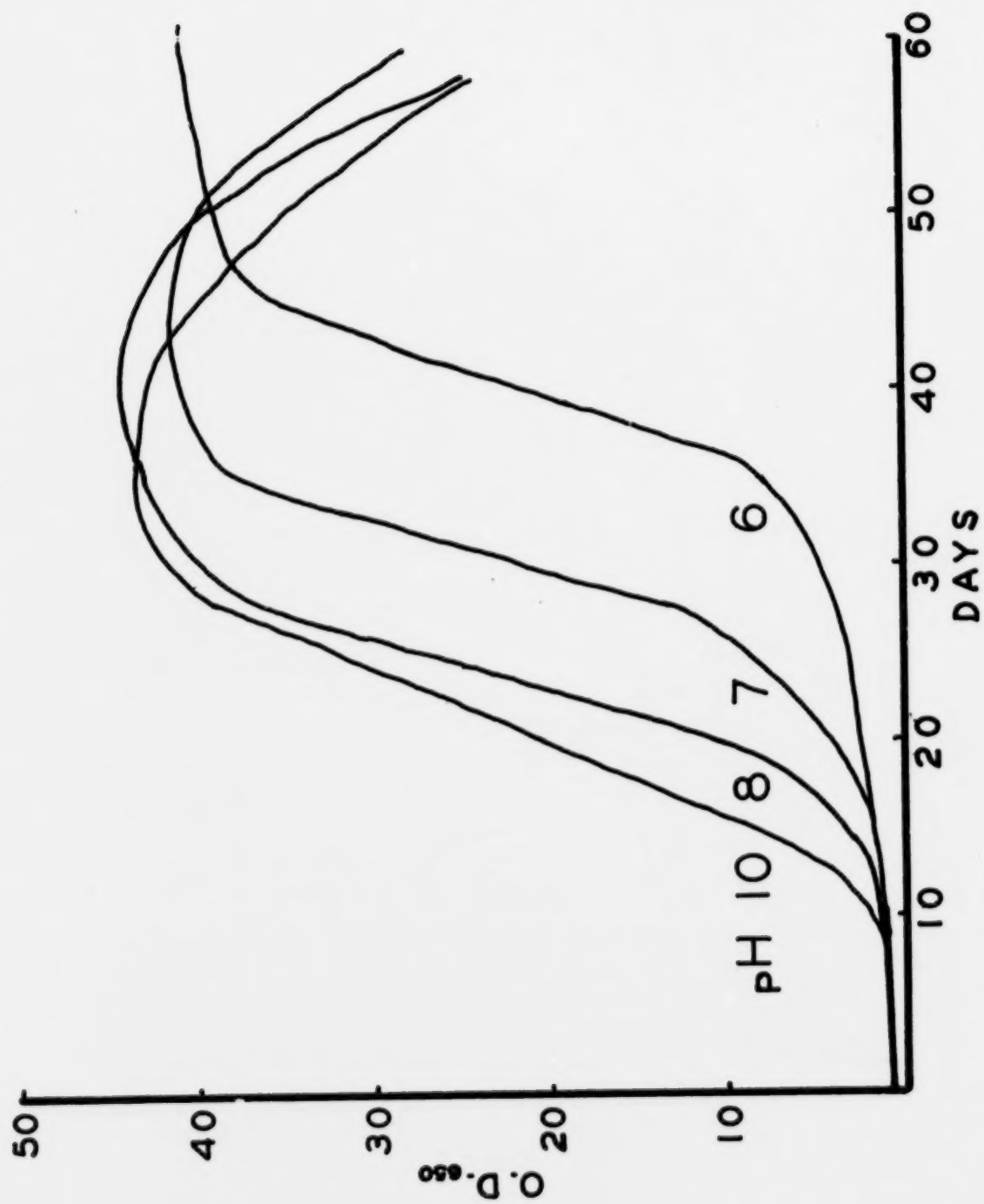


Figure 2. Growth of *Oscillatoria agardhii* (strain E10) in ASM medium with varying initial pH values.

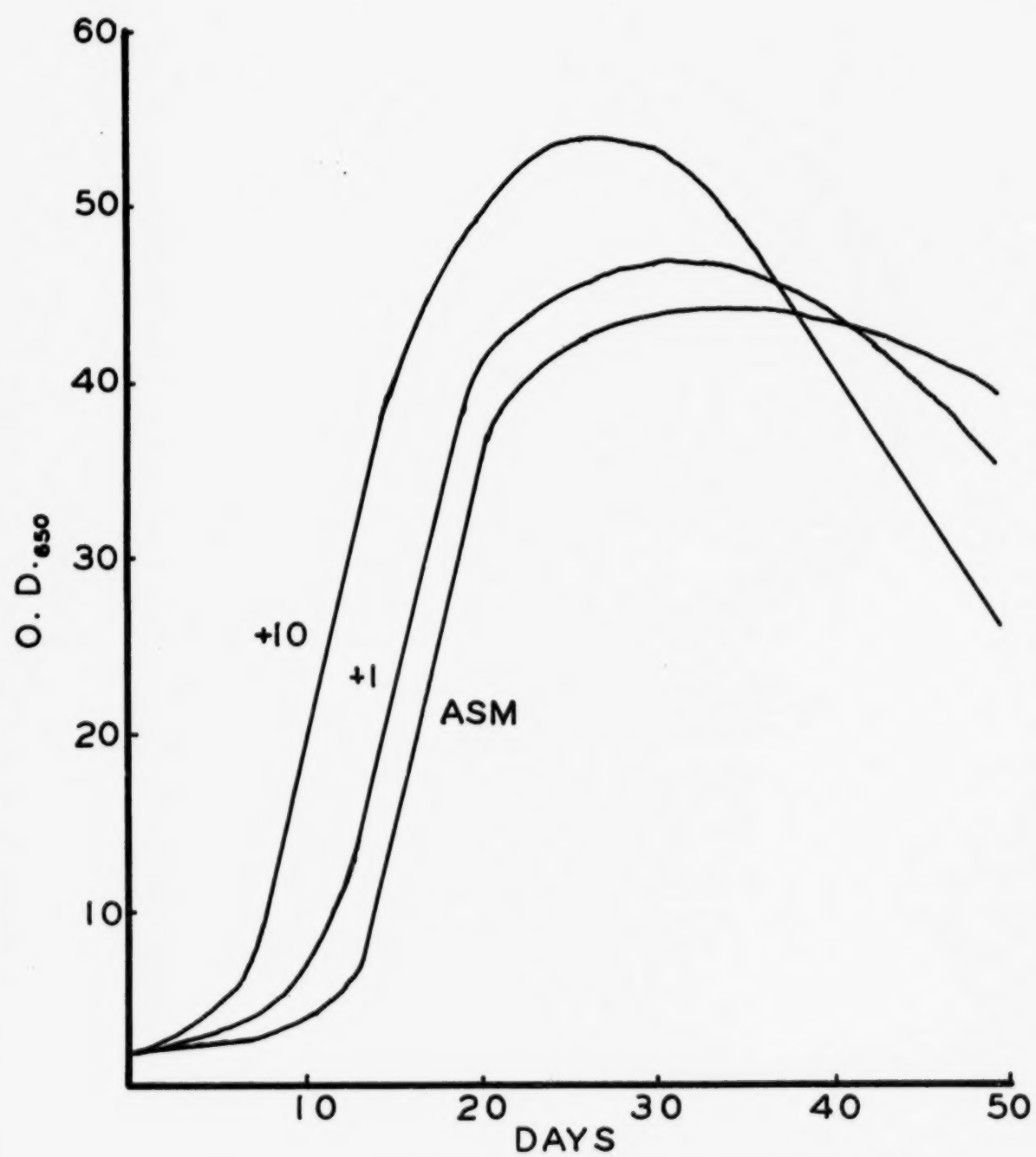


Figure 3. Enhancement of growth of *Oscillatoria agardhii* (strain E10) in ASM medium with the addition of soil extract.

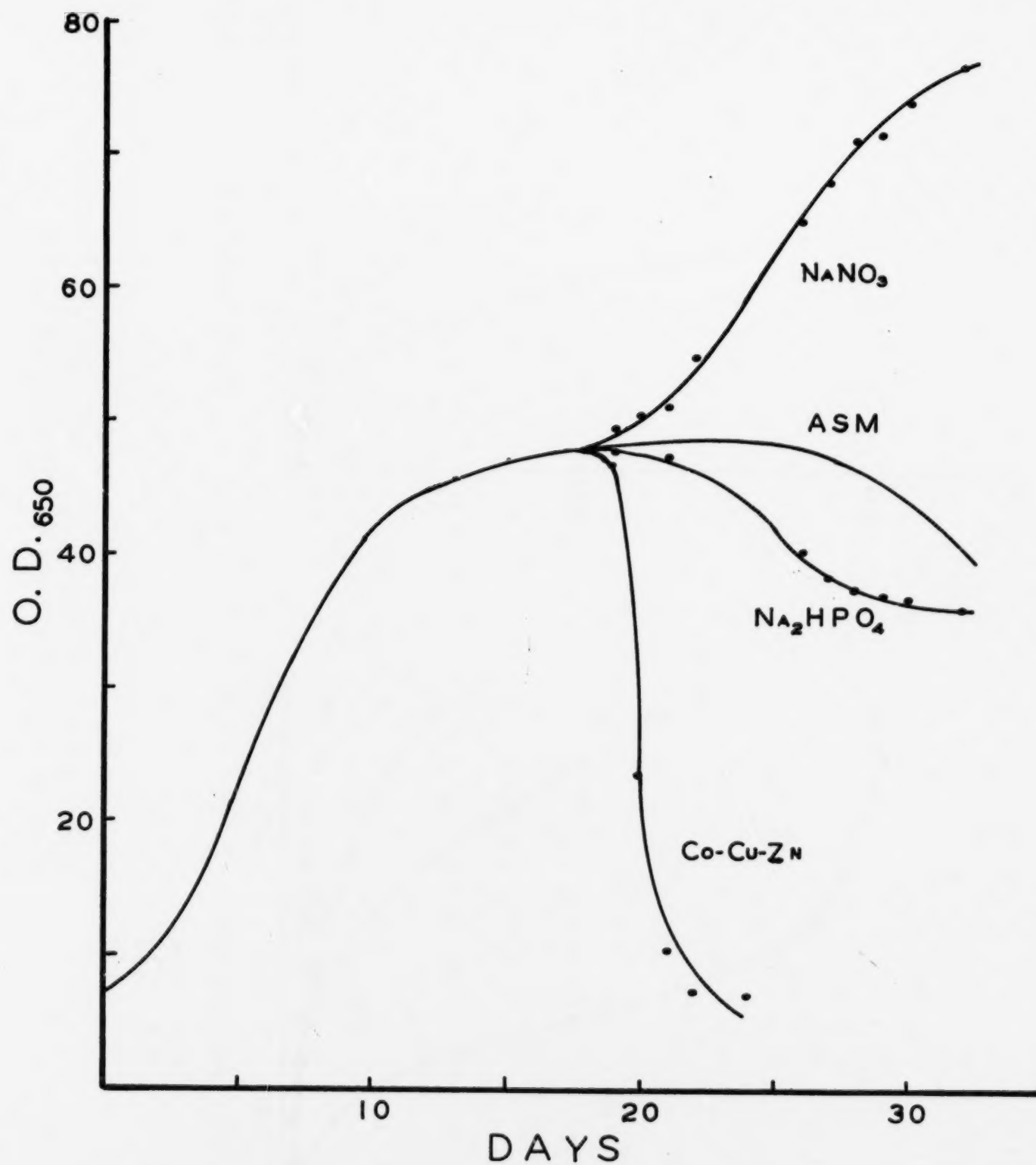
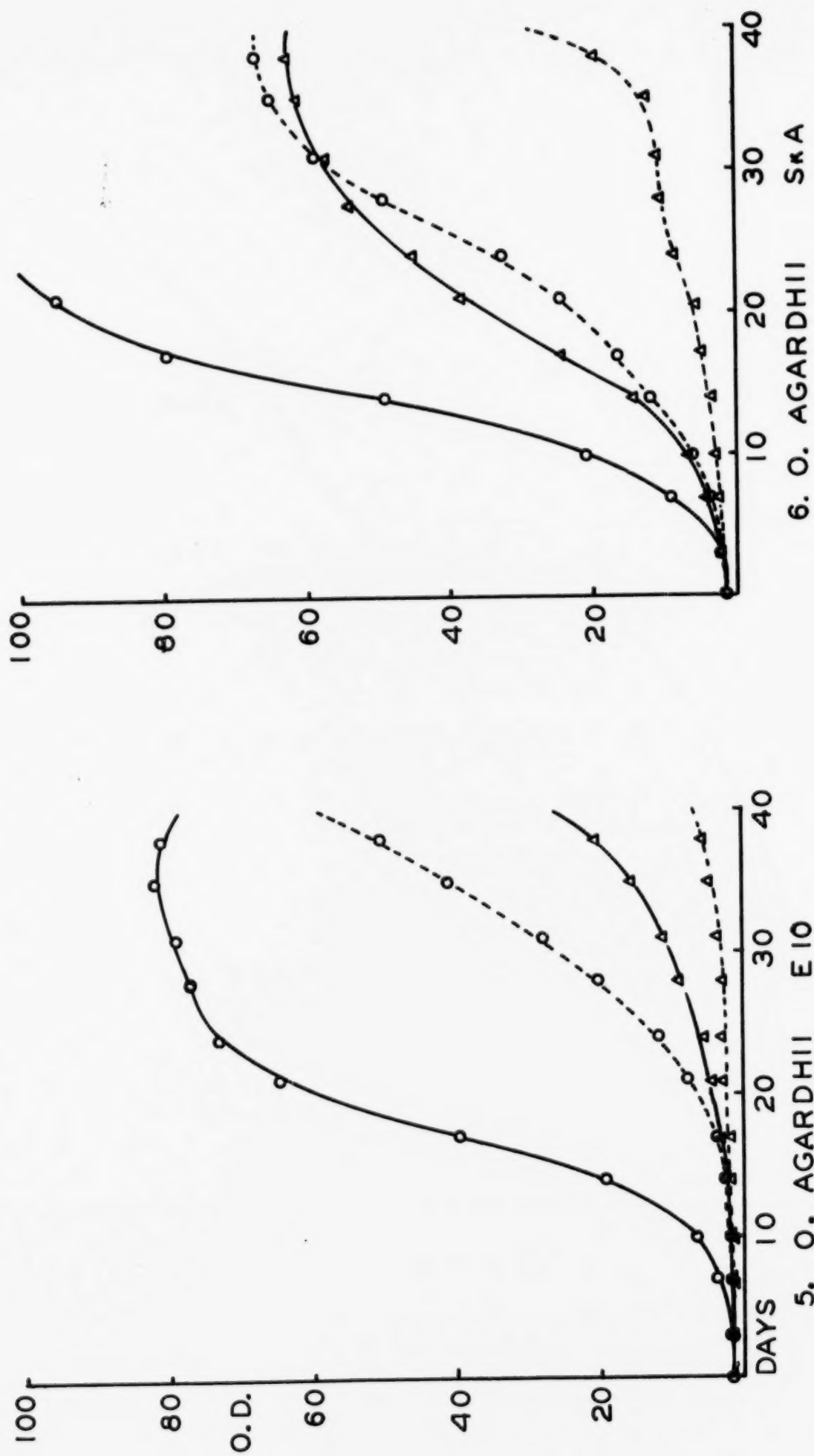
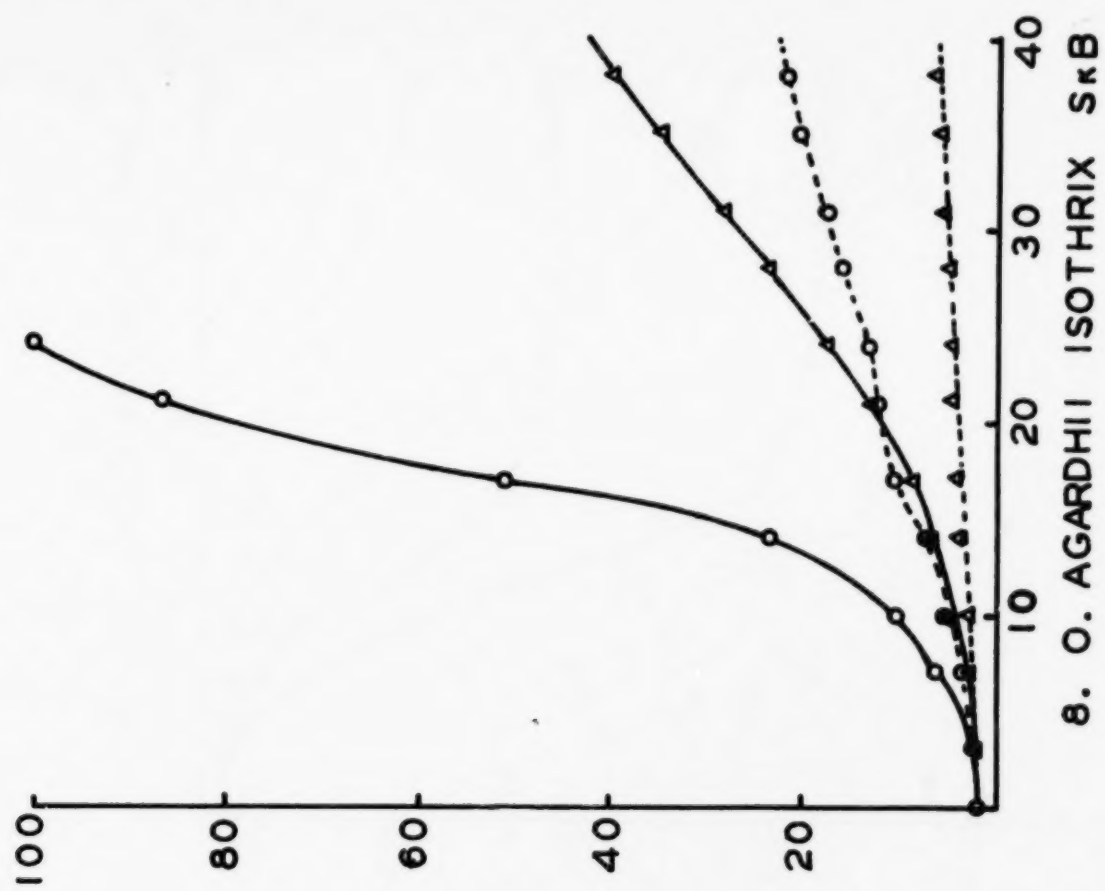
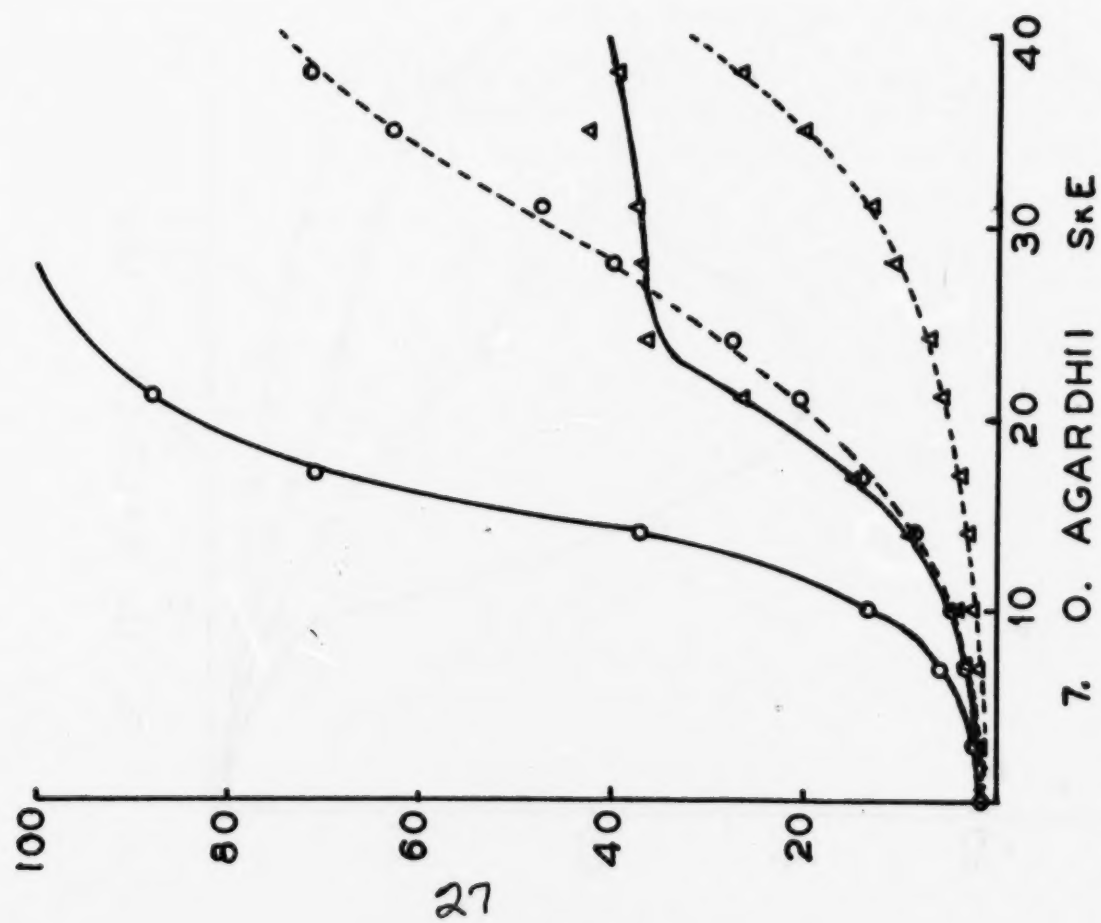


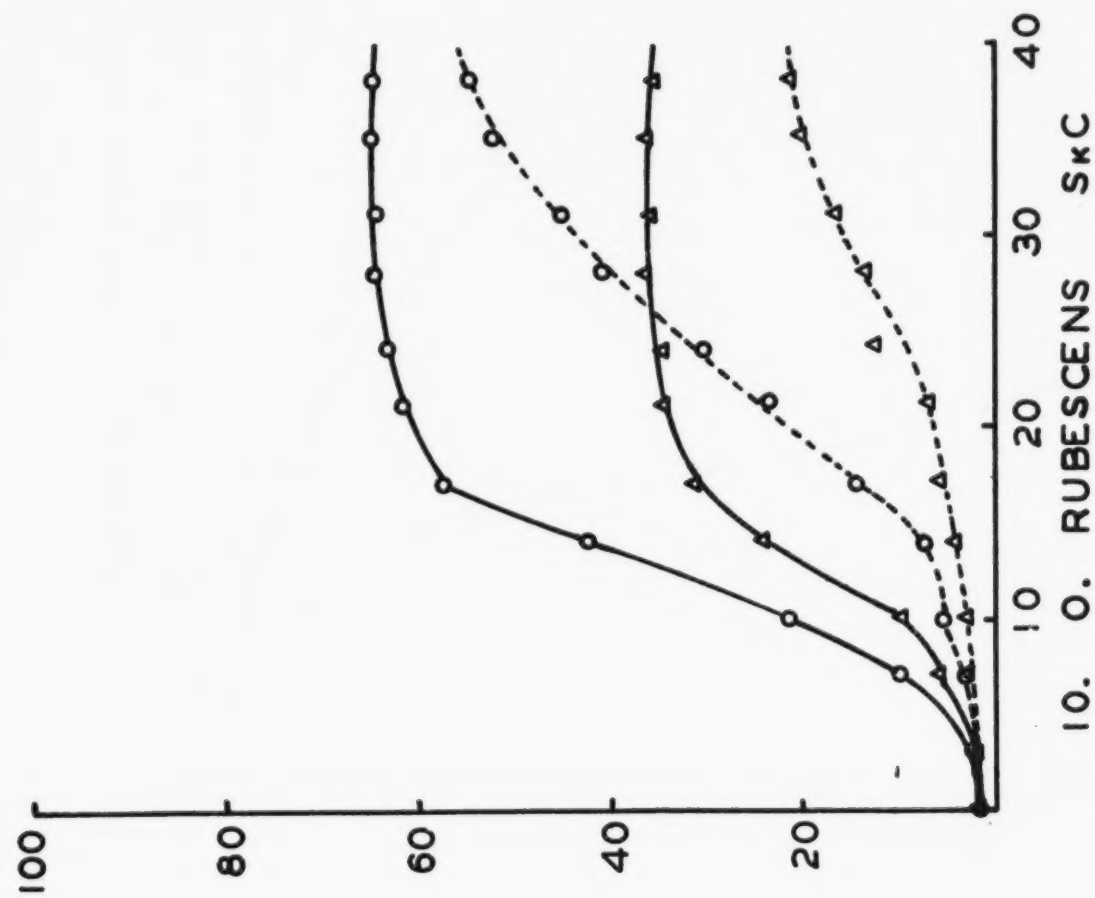
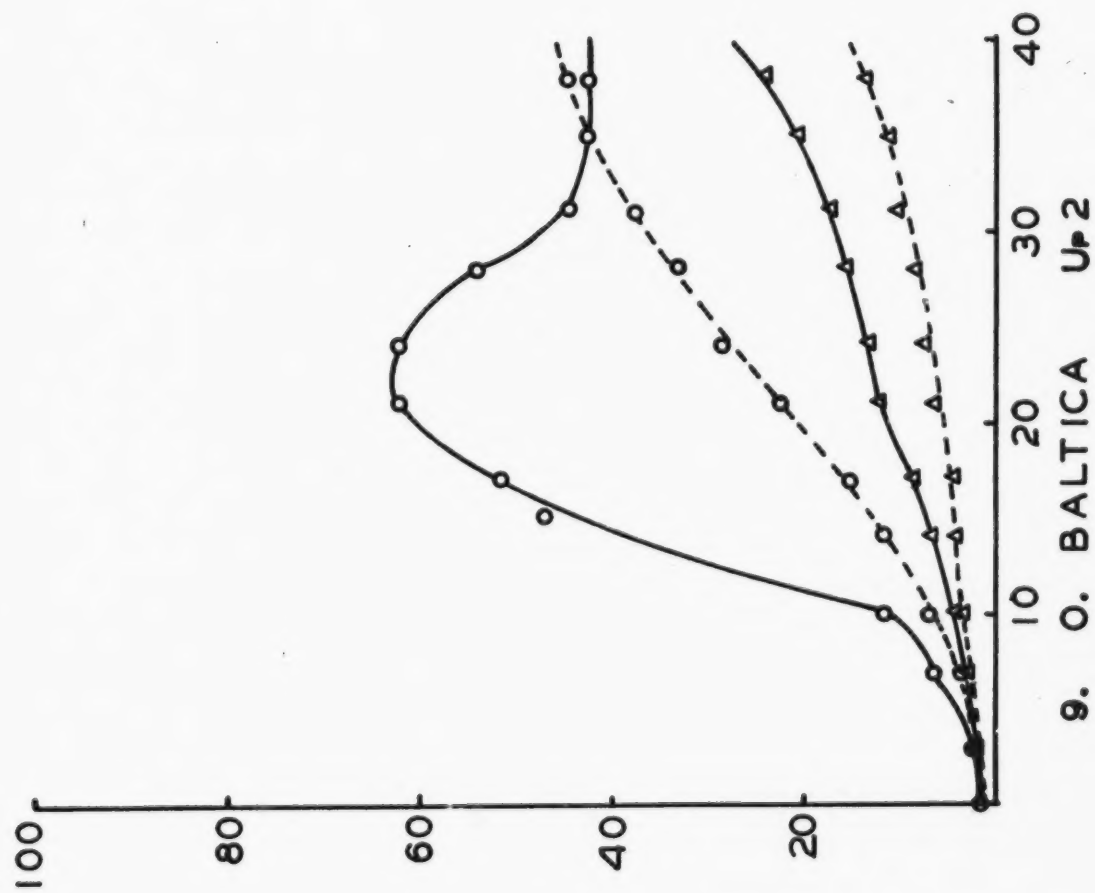
Figure 4. Effects of the addition of increased amounts of nutrients during the stationary phase of the growth of Oscillatoria agardhii (strain E10) in ASM medium.



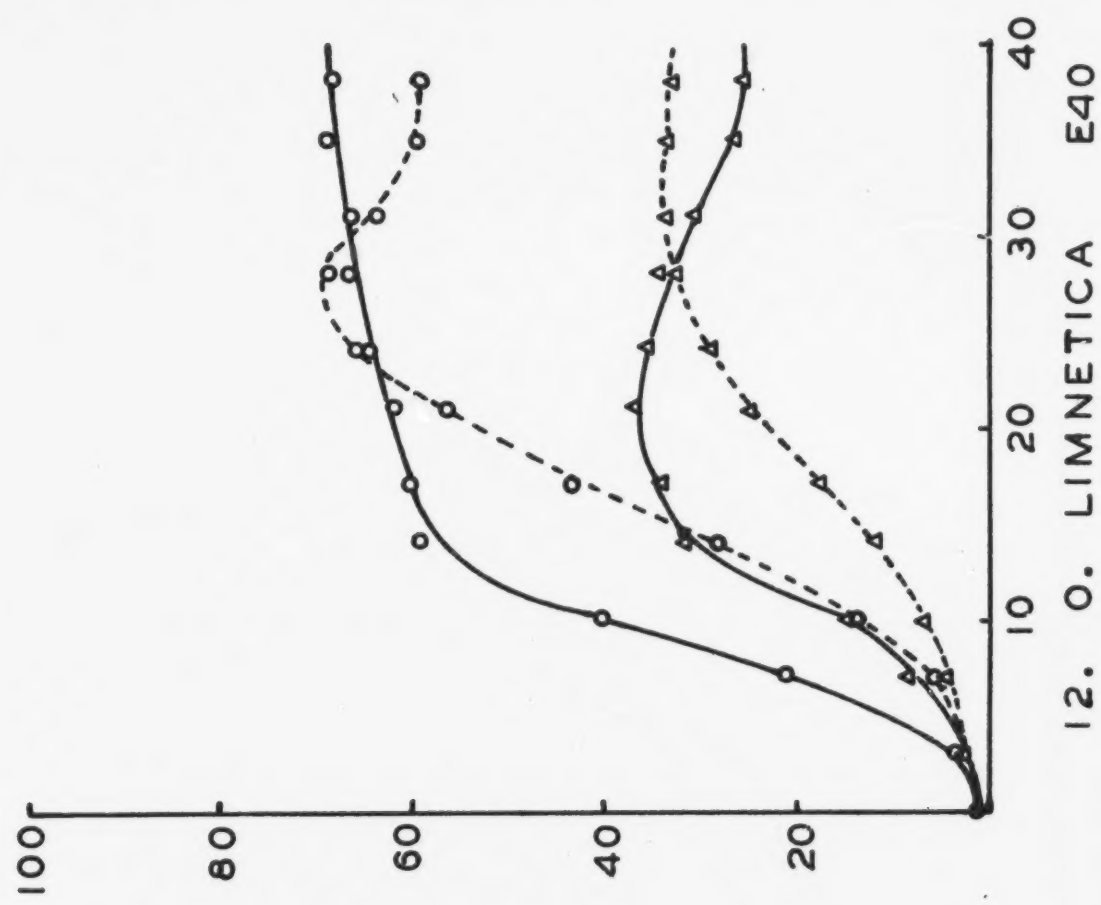
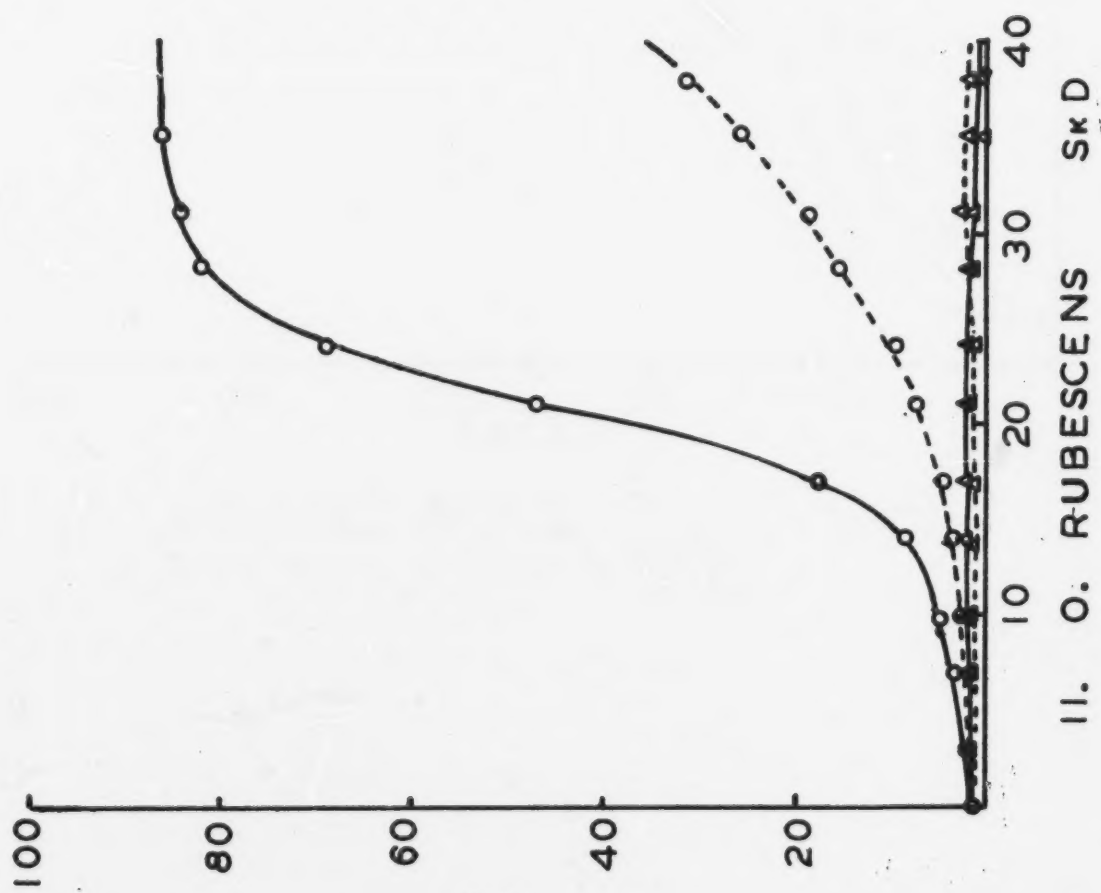
Growth response curves for various blue-green algae grown in ASM and ASM-1 media at 15° and 20°.



See Figure 5 for legend.



See Figure 5 for legend.



See Figure 5 for legend.

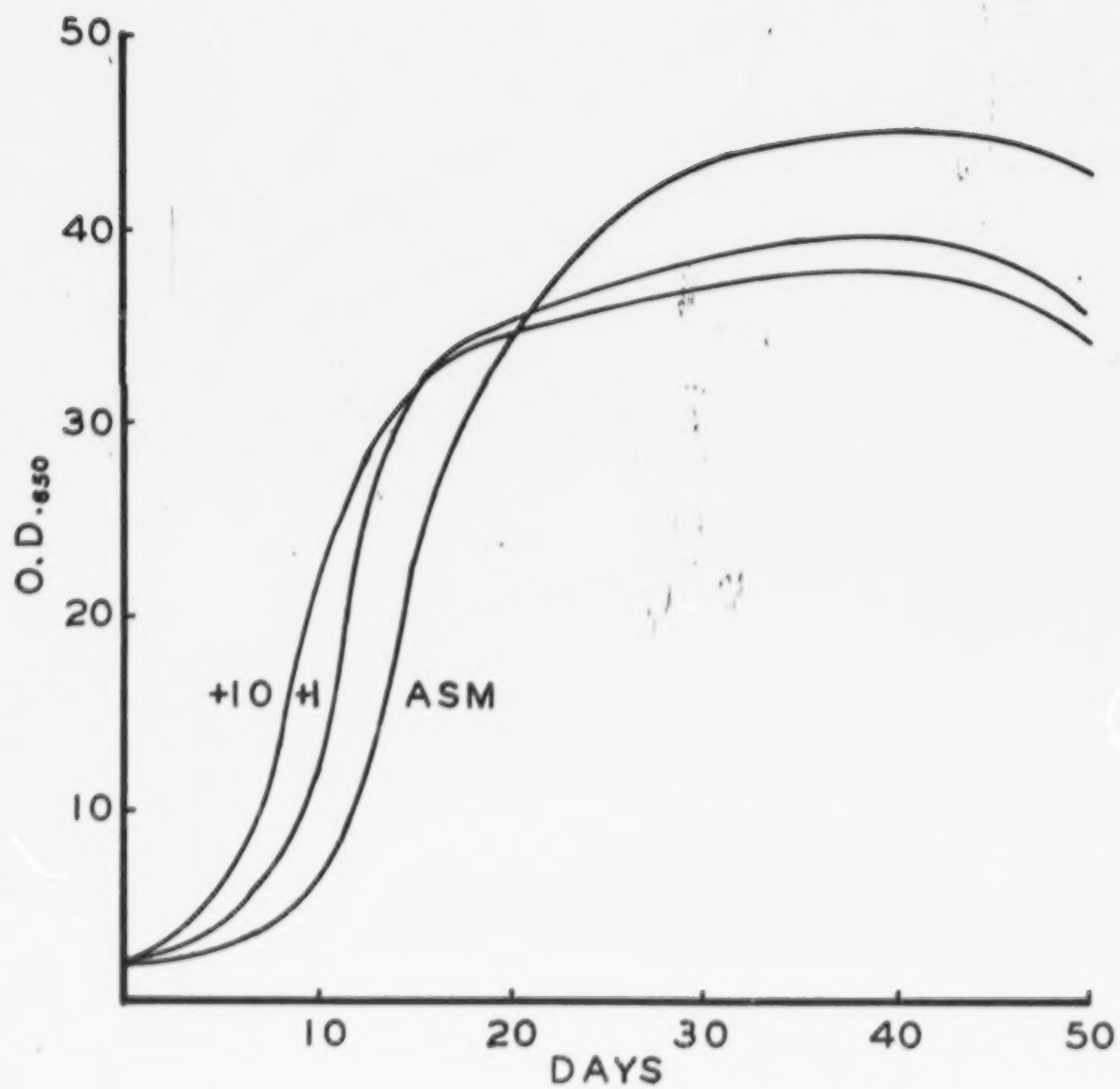
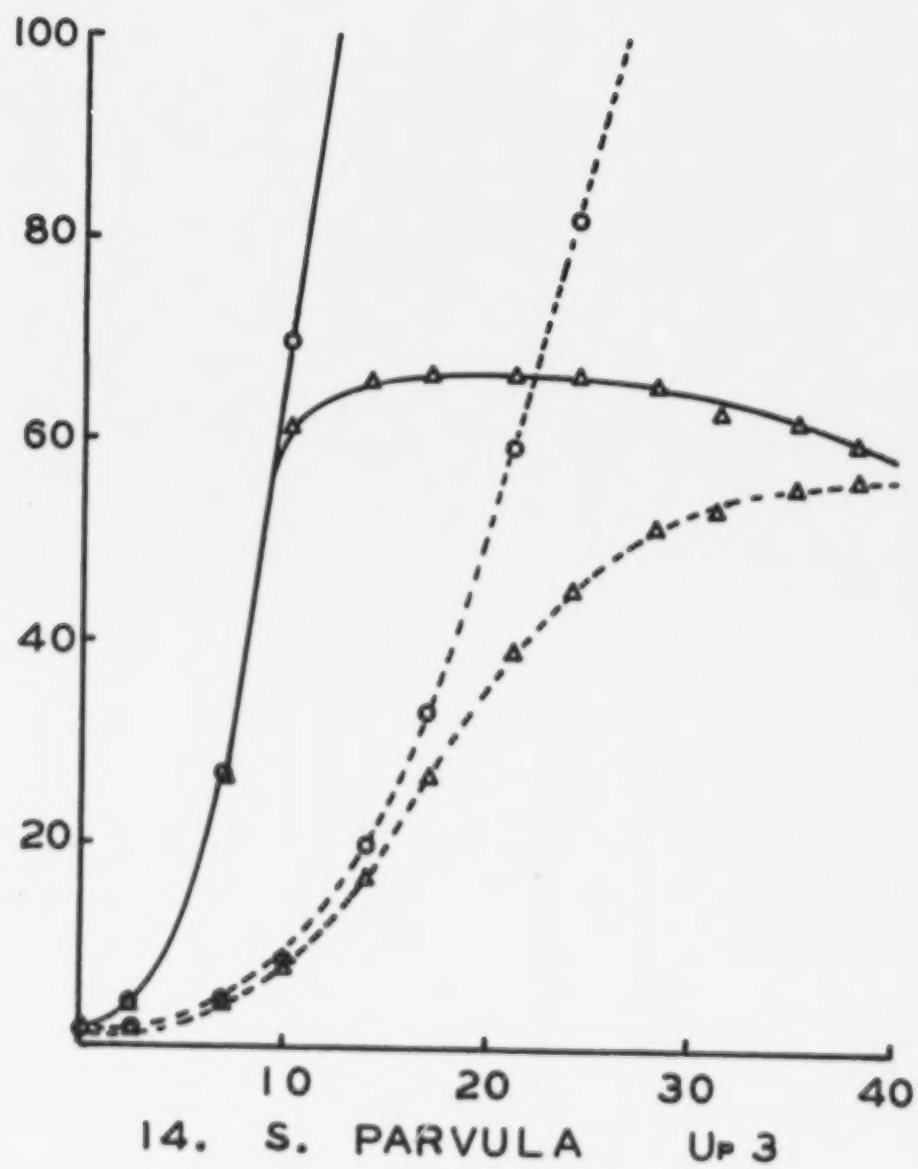


Figure 13. Enhancement of growth of *Oscillatoria rubescens* (SkC) in ASM medium with the addition of 1% and 10% treated sewage effluent.



See Figure 5 for legend.

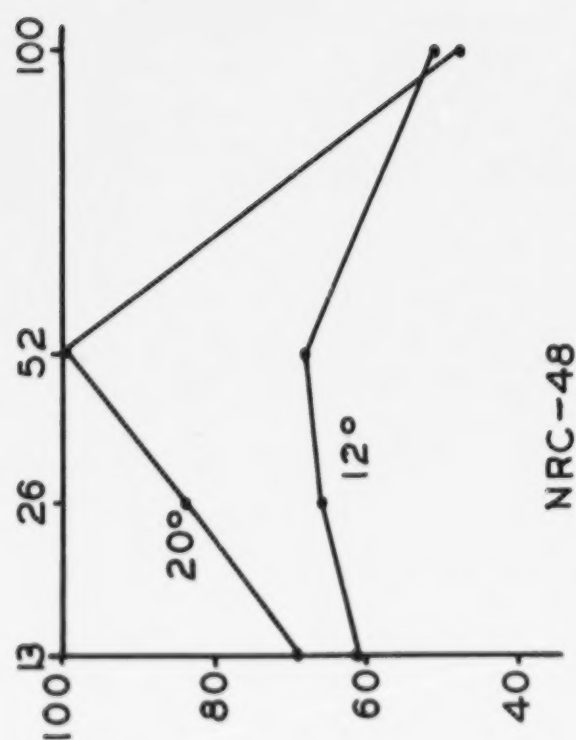
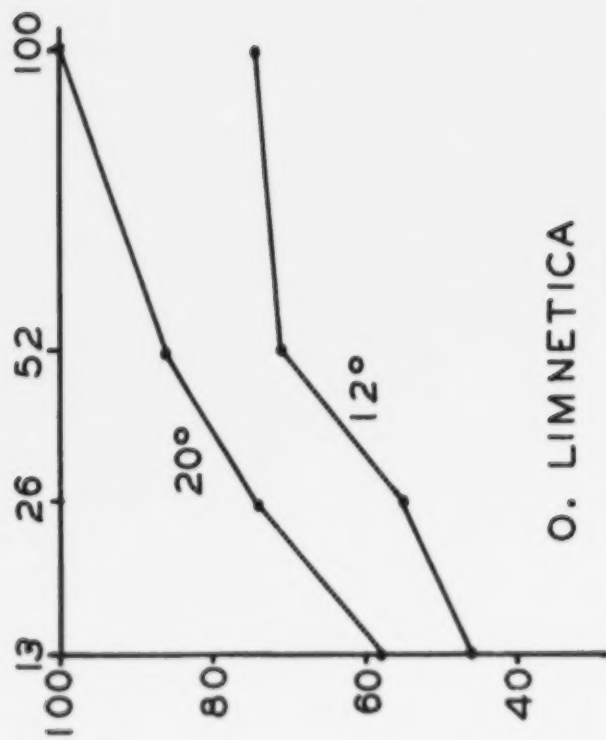
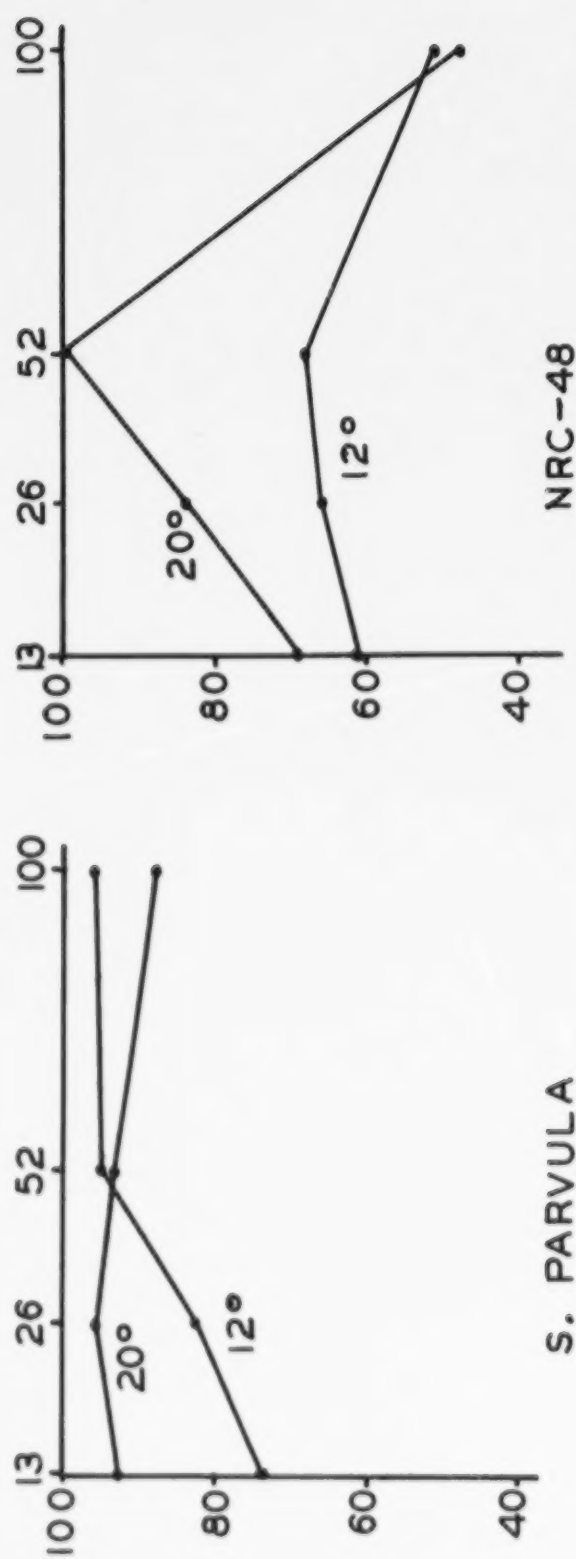
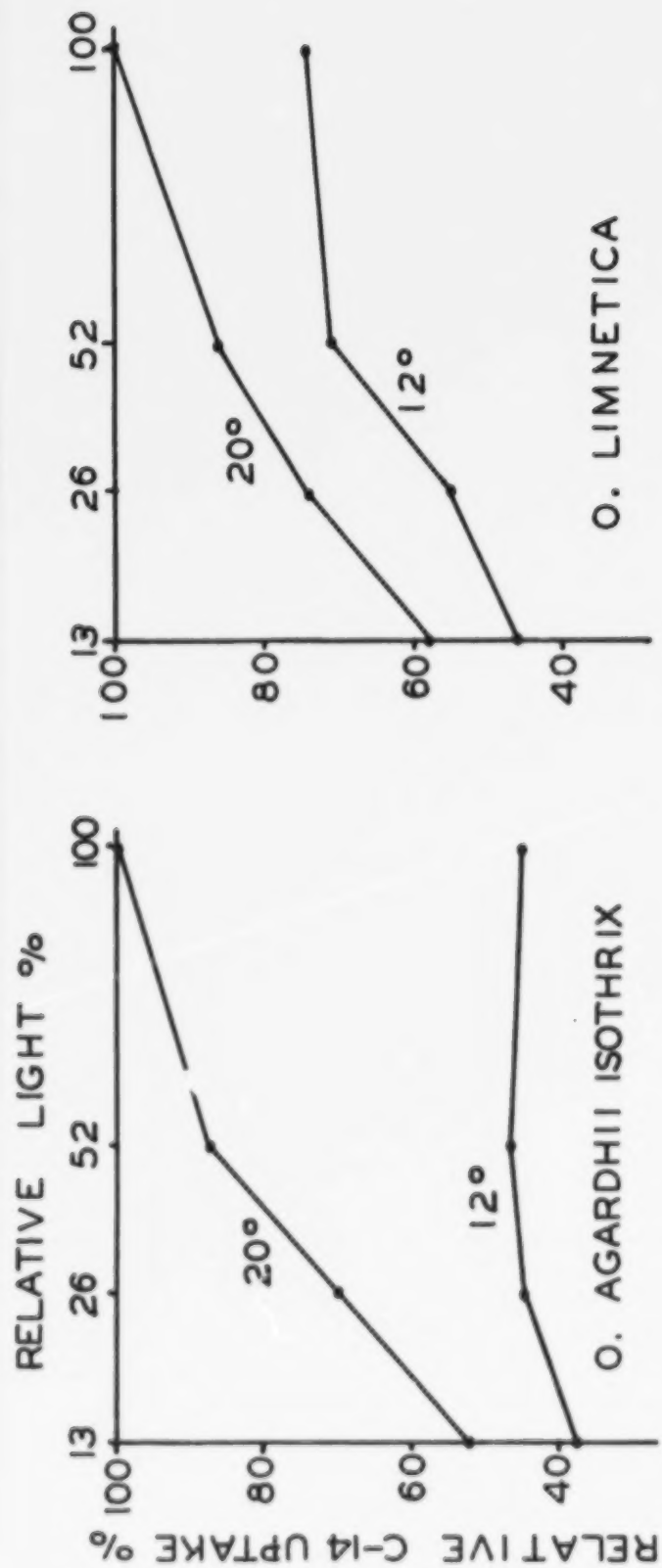


Figure 15. Complementary relationship of the effect of light intensity and temperature on C-14 uptake of four blue-green algae in ASM medium.

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ASPECTS OF THE NITROGEN NUTRITION OF SOME
NATURALLY OCCURRING POPULATIONS OF BLUE-GREEN ALGAE

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INTRODUCTION

Although nitrogen is a key element required by living organisms and its abundance in natural systems is vitally important in problems of aquatic productivity, little is actually known about its role in lacustrine systems. The study of the ecological role of nutrients is still in its infancy, with a great need for the development and evaluation of techniques. A perusal of Hutchinson's (1957) chapter on the nitrogen cycle in lakes shows that, although a fair amount of information exists concerning levels of nitrogen compounds and gross nitrogen balance in lakes, there is much less on the actual assimilation of nitrogen sources by algae; and that, of the information available, a large part is derived from laboratory studies.

The cycle of a nutrient in nature is always a complex process, with many organisms contributing to the transformation. The bacterial population is especially important, since the "feed rate" of a nutrient in lakes is at least in part the result of the heterotrophic breakdown of organic material by these organisms. This in situ regeneration is one of the aspects of the nitrogen cycle which has been largely ignored, especially in considering the nutrient sources available to the photosynthetic population in the euphotic zones of lakes. Nutrient cycling in its most direct pathways of uptake, release, and regeneration has thus represented a "black box" situation and yet Riley (1951) has suggested that regeneration in the upper 200 meters accounts for 90% of the organic matter produced annually in the oceans. Grill and Richards (1964) have measured the release of nitrogen, phosphorus, and silica from a diatom population in a darkened polyethylene drum. Although the release of ammonia was observed, there appeared to be no nitrification judging by the nitrate accumulation.

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Although this discussion has had no direct relationship to blue-green algae, it has been included to show the problems which must be faced when considering the nutrition of naturally-occurring populations. For this reason, we have chosen a direct approach and have attempted to measure the actual utilization rates of nitrogen compounds in the water; simultaneously, we have tried to assess the magnitude and importance of in situ regeneration of these compounds. Blue-green algae play a very important role in the nitrogen cycle of lakes, and the behavior of these parameters during blue-green algal blooms has been interesting. My discussion will center around such populations; but more specifically, I will emphasize nitrogen-fixing blooms of planktonic, blue-green algae.

NITROGEN FIXATION BY BLUE-GREEN ALGAL BLOOMS

Fogg and Stewart (1965) have recently reviewed the current status of ecological information on the role of nitrogen fixation by blue-green algae. The role of planktonic blue-green algae in the fresh water nitrogen cycle has attracted interest especially because of the unique ability among algae of members of this group to fix atmospheric nitrogen. This is believed to account for the observation that although Myxophyceae are known to have a high nitrogen content on a unit weight basis, they are often found in waters containing little combined nitrogen. Certainly, a major role for Anabaena has been well documented. Hutchinson (1957) attributed the addition of 490 mg m³ of nitrogen to a bloom of Anabaena in Linsley Pond, and the work of Prowse and Talling (1958) on a reservoir in the White Nile has indicated that there Anabaena is important in determining the seasonal succession of algae. A bloom of Anabaena during the low nitrogen period following impoundment was followed by a population of algae which were apparently using nitrate regenerated from the nitrogen fixed by the initial population of blue-green algae. This not only confirms the possible importance of nitrogen-fixing blooms of algae, but suggests that nitrogen assimilated into an algal population can become available rather quickly to a subsequent population, presumably as a result of the release of ammonia upon the death of the cells. The implications for the control of population succession are obvious. Although the evidence for nitrogen fixation was not direct in any of these cases, the indirect evidence supports the conclusion that major nitrogen fixation occurred in connection with these populations. In each case, large numbers of organisms with high nitrogen content grew without any apparent adequate nitrogen source; and in each instance, the population consisted predominantly of members of a genus known to fix nitrogen in pure culture. Indeed, many of the constituent species have been critically tested--for instance, Anabaena flos-aquae (Gorham, et al., 1964) and Anabaena cylindrica (Fogg, 1942). Both these species are frequent components of planktonic freshwater blooms. There are other examples in which nitrogen fixation is strongly suggested for

algal blooms, and it is sufficient to say here that the circumstantial evidence has been convincing.

We have made measurements of nitrogen fixation rates using ^{15}N in Sanctuary Lake, a temperate reservoir, and found high rates of fixation, having fortuitously selected a lake in which nitrogen fixation is an important feature during much of the summer. A positive correlation between fixation rates and light was found, such that after a period of darkness the fixation rates became very low. Clearly the algae were involved directly or indirectly. The population in this lake was diverse, but dominated by *Anabaena* sp. during the periods of high nitrogen fixation. Goering and Neess (1964) found light-dependent fixation at fairly high rates in one of the Madison, Wisconsin, lakes. The work of Stewart (1962, 1964) has provided information on nitrogen fixation, both in the laboratory and *in situ*, by marine blue-green algae from the water and from a sand dune slack; and Goering, Dugdale, and Menzel (1966) have measured significant nitrogen fixation in connection with populations of *Trichodesmium* in the open ocean. The close interrelationship between nitrogen fixation and photosynthesis (Fogg and Than-Tun, 1960) results in a readily available source of energy for nitrogen fixation by blooms in the euphotic zone of a lake. This fact, coupled with the potential independence of such populations from the nitrogen nutrient levels in the water, may allow nuisance blooms to develop under conditions unsuitable for non-nitrogen-fixing algae. Consequently, the environmental requirements for nitrogen-fixing blooms of algae pose an important problem. Many bloom-forming blue-greens are not among the nitrogen-fixers and, of course, these also play an important role in the phytoplankton communities of lakes.

METHODS

Although well-established limnological techniques were used to obtain most of the information reported here, some of the experimental techniques need a brief explanation.

The ^{15}N methods have been described in the literature (Neess et al., 1962; Dugdale and Dugdale, 1965). In the method for nitrogen fixation, dissolved nitrogen is removed from the sample by sparging at reduced pressure with a helium-oxygen or helium-oxygen-carbon dioxide mixture, and is then replaced by nitrogen containing a known enrichment of $^{15}\text{N}_2$. For uptake measurements of nitrate and ammonia salts, ^{15}N enriched compounds are simply added to the water sample enclosed in a bottle. Incubation may either be carried out *in situ* or under standard conditions, and afterwards the ^{15}N enrichment of the particulate fraction, which contains largely algae, is determined.

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The isotope dilution method for measuring the rate of supply of ammonia into the dissolved ammonia fraction of the water uses ^{15}N ammonia, which is added to a sample of lake water. The system is equilibrated and immediately an aliquot is filtered through a glass filter; the isotope ratio of the ammonia fraction in the filtrate is determined following vacuum distillation and conversion to N_2 using alkaline hypobromite. This initial aliquot gives a value for the ammonia concentration in the water and also a zero time value for the isotope ratio. Subsequently, aliquots filtered at regular intervals and treated similarly give a rate of dilution of the added labeled ammonia by unlabeled ammonia from another nitrogen fraction in the water.

Quantitative estimates of the algal population have not been made, although Lugol's acetic acid preserved samples are available for each experimental day. Usually, chlorophyll *a* or total chlorophyll estimates, along with determinations of the particulate nitrogen content of the water, have been used as an estimate of algal population, with only qualitative information on the composition of the populations. Particulate nitrogen is a particularly useful parameter to measure for this type of study, and can be determined with high precision using a Coleman Nitrogen Analyzer on material collected on a glass filter.

NITROGEN METABOLISM DURING THE ANNUAL SMITH LAKE ANABAENA BLOOM

We have studied Smith Lake, a small lake in the Tanana River Valley of Central Alaska, near Fairbanks. Four years of work are now completed on this lake. The major annual bloom in this lake is dominated by *Anabaena flos-aquae*, and it typically lasts only two weeks.

The conditions preceding the bloom are highly reproducible from year to year, probably because of the climatic conditions peculiar to the subarctic. The most remarkable feature, apart from the long severe winter, is the absence of a normal spring. This is because break-up, (which refers to the melting of the snow and ice on the lakes and surrounding terrain), corresponds to a period of long daylight hours and intense insolation. The temperature rise of the water upon break-up is spectacular, and it is during this period of rapid temperature increase that the *Anabaena* population builds up. Thus, temperature may be of direct significance in this precipitous population increase. Under these conditions, a lake only three meters deep can become stably stratified and resist complete mixing for most of the summer in spite of considerable winds. Dilute ice-melt water may then float over the water which has remained unfrozen during the winter. This happens in Smith Lake, which has a maximum depth of three meters and an estimated mean depth of two meters. Examples of the depth distribution of temperature during the bloom period are shown in Figure 1. The relationship between bloom

Aspects of Nitrogen Nutrition of Some Blue-Green Algae

formation and temperature can be seen in Figure 2, in which surface temperature and particulate nitrogen are plotted simultaneously. Table 1 shows some physical and chemical properties of the lake during the bloom.

The nutrient content of the water at this time is strongly influenced by the biological events preceding the ice breakup. February, March, and April are characteristically months of complete anoxia in the water, and no algae are present. In May, a population of μ -flagellates and small green cells appear and are apparently influential in terminating the anoxic status of the water. There is, as yet, no contact of the water with the atmosphere, but considerable light penetrates the ice allowing quite high rates of photosynthesis. This population is influential in reducing the very high nutrient content of the water and, indeed, the population decline corresponds to the time when the 40 μ g-atoms of ammonia present have been completely removed into the particulate nitrogen fraction. Nitrate is not present, having been lost by denitrification during the anoxic period (Goering and Dugdale, 1966). The Anabaena population which follows develops at a time when the nutrient content of the water is low but beginning to build up.

The Anabaena population is virtually unialgal and builds up at a very rapid rate exhibiting high rates of photosynthesis in the surface water. In Figure 3, we can see the chlorophyll a content of the water during a typical summer season. The first peak represents the under-ice population, and the second, the Anabaena. Figure 4 shows the seasonal distribution of photosynthesis measured by the ^{14}C method. The two major periods of blue-green algal dominance are the high peak (Anabaena), and the lower, but steady, mid-summer production during which time Aphanizomenon is the most abundant form. The extremely high rate of photosynthesis at the June peak, when sustained for virtually 24 hours, allows the tremendously rapid growth in population. I am deliberately including data from several years in the figures in order to emphasize the annual precision in timing.

Measurements of the nitrogen uptake by this bloom show that in the early phases, ammonia is the predominant form used, with nitrate second. Nitrogen fixation rates are low during this period, and initially the bloom seems to build up on ammonia. The source of this ammonia was unclear and isotope dilution experiments were carried out to see whether the rate of ammonia supply within the water was adequate to allow the uptake rates measured without assuming advection. Table 2 shows the results of these determinations. Although the precision of the experimental procedure is not very good yet, these results are reliable in approximate magnitude. The value for June 3 indicates that the supply rate is more than adequate for the uptake rate measured, which agrees with the analytical observation that the ammonia concentration is increasing in the water. The extremely high rates of supply measured following

ENVIRONMENTAL REQUIREMENTS OF BLUE-GREEN ALGAE

Table 1

Some physical and chemical properties -
Smith Lake *Anabaena* bloom

1964

Depth (m)	pH	Alkalinity	O ₂ mg liter ⁻¹	T °C	Specific cond. μHmos/cm ²	nutrients (μg-at liter ⁻¹)		
		mg liter ⁻¹ CaCO ₃				NO ₃ ⁻ -N	NH ₄ ⁺ -N	PO ₄ -P
<u>30 May</u>								
0	7.10	18.0	-	13.5	60.2	0.1	0.4	2.2
1	7.10	23.0	-	5.6	52.8	0.0	-	1.7
2	6.90	40.0	-	5.2	154.0	0.5	-	3.5
<u>16 June</u>								
0	9.35	-	11.5	20.4	75.8	0.2	2.8	0.6
1	9.00	-	10.2	18.6	77.2	0.2	1.5	0.2
2	6.91	-	1.7	10.2	103.0	0.6	7.7	2.1
<u>18 June</u>								
0	7.97	27.0	8.7	22.2	83.9	0.1	-	-
1	7.65	27.7	6.4	18.7	85.1	0.1	-	-
2	6.87	34.3	1.2	10.7	113.4	0.4	-	-
<u>22 June</u>								
0	7.34	30.0	6.7	18.8	76.4	0.3	16.5	0.7
1	7.26	29.0	6.4	18.8	80.3	0.3	-	0.9
2	6.93	40.0	6.2	9.6	110.5	0.4	-	2.6
<u>13 July</u>								
0	7.45	28.0	7.6	19.7	95.9	-	5.8	-
1	7.27	28.1	7.2	18.0	97.5	-	-	-
2	6.99	28.0	1.9	15.1	115.5	-	-	-

the bloom are surprising; much of this ammonia must be taken up by the macrophytes which show tremendous development at about this time. This does seem to confirm the immediate availability of nitrogen incorporated

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in a bloom. The rate of supply of ammonia from the previous population is also apparently adequate to initiate the rapid growth of Anabaena in spring.

Table 2

Results of ammonia uptake and ammonia supply experiments. Smith Lake, Alaska

Date	NH ₄ ⁺ -N $\mu\text{g liter}^{-1}$	*PN $\mu\text{g liter}^{-1}$	Uptake rate $\mu\text{g liter}^{-1}\text{hr}^{-1}$	Supply rate $\mu\text{g liter}^{-1}\text{hr}^{-1}$
3 June 1965	28.3	371.6	7.0	13.3)
23 June 1965	361.2	102.3	16.4	343.1) <u>Anabaena</u> bloom
21 July 1965	29.4	88.9	1.5	2.9
13 Aug. 1965	20.3	81.2	2.1	2.8
31 Aug. 1965	47.4	64.5	0.5	12.8
30 Sept. 1965	31.5	77.8	0.7	7.6
20 Jan. 1966	227.5	62.7	0.6	100.1
3 Feb. 1966	338.8	110.6	0.0	67.8
15 Feb. 1966	319.9	104.2	8.2	16.0)
15 March 1966	515.8			107.3) anoxic
22 April 1966	676.1	105.9		123.7)

*Particulate nitrogen

Table 3 shows the uptake rates of ammonia, nitrate, and molecular nitrogen during the bloom. A progressive shift is evident from a very high ammonia utilization rate to a relatively greater importance for nitrogen fixation which, at one point, accounts for half the nitrogen assimilated. The highest absolute rates of nitrogen uptake, taking into account all three forms measured, are found early in the bloom, during the time when the population is increasing at its maximum rate. This rate approaches 24 $\mu\text{g liter}^{-1}\text{hr}^{-1}$. During the nitrogen-fixing phase, the algae become noticeably concentrated near the surface, resulting in very high photosynthetic rates on a unit volume basis of surface water. This concentration can be seen from the chlorophyll data shown in Table 4. As a result of this intense activity at the surface, there is an abrupt rise in pH near the surface, which lasts perhaps one or two days. This is a unique and rather closely-timed annual event. The annual pH curves for Smith Lake surface water during three years are shown in Figure 5. Shortly after this, the population of Anabaena declines.

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Table 3

Uptake of NH_4^+ -N, NO_3^- -N, and N_2 -N
during the spring *Anabaena* bloom, Smith Lake

Date	Particulate Nitrogen $\mu\text{g liter}^{-1}$	$\mu\text{g liter}^{-1} \text{ hr}^{-1}$			
		NH_4^+ -N	NO_3^- -N	N_2 -N	ΣN
4 June 1964	125.3	0.82	0.36	.02	1.20
6 June 1964	263.6	17.23	4.88	1.76	23.87
8 June 1964	358.8	5.99	4.12	2.45	12.56
10 June 1964	405.4	6.41	0.75	2.88	10.04
12 June 1964	640.1	8.21	0.37	2.28	10.86
15 June 1964	753.1	1.70	0.15	0.19	5.10
23 June 1964	--	0.94	0	0.02	0.96
25 June 1964	71.3	--	--	--	--

Table 4

Depth distribution of chlorophyll *a* during *Anabaena* bloom, 1964

Depth (m)	8 June	10 June	12 June	18 June	30 June
		$\mu\text{g liter}^{-1}$			
0.0	22.6	17.1	132.3	22.2	7.7
1.0	10.7	34.7	19.4	18.7	8.2
2.0	10.7	6.8	-	10.7	3.2

The filaments composing the bloom undergo changes in appearance as the population progresses through its growth and decline. At first, the filaments are long, somewhat coiled, with very even, healthy green cells. Initially there are no heterocysts, but these develop as the nitrogen fixing activity of the algae increases. Akinetes appear at about the time of the pH rise, and following this there is clumping and disintegration of the filaments, so that free akinetes and heterocysts are visible in the water. Following this, there are no *Anabaena*

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in the water. The cause of the decline is an interesting problem. Perhaps the pH has a role, either directly or indirectly by causing CO₂ limitation, or perhaps by increasing the strength with which a metal ion is bound to an organic chelating agent. The water is strongly colored by a brown organic material, which behaves in a manner similar to that described by Shapiro (1964). The influence of pH in increasing the strength with which cations are bound could be important where high growth rates are causing rapid removal of a metal.

OTHER BLUE-GREEN ALGAL POPULATIONS

Following the Anabaena bloom decline in Smith Lake, Aphanizomenon becomes the dominant form. The nutrient levels never become very low, but rather remain at a constant level, and the growth of the population appears to be controlled by grazing, with a very dense population of zooplankton present now. The diversity in algal forms present is much greater, and nothing approaching a unialgal population is found. Anabaena appears again in this mixed population, with very long filaments composed of healthy green cells. The macrophyte population is building up fast at this time, and must continue to drain considerable quantities of nutrients from the water. Ammonia is predominantly used by the phytoplankton, and although nitrogen fixation was detected at one time during later summer, significant rates were never found except in connection with the spring Anabaena bloom.

An Aphanizomenon-dominated population is found in a second small lake only two miles from Smith Lake. This lake is similar in surface area and in the strong brown coloration of the water, but differs in depth, being 6 meters deep at the maximum depth found. Nitrate accumulates in this lake in spite of much of the water remaining anoxic during the entire summer--I do not know why this nitrate is not denitrified under these conditions. Water below 2.5 meters remains anoxic during the summer. The Aphanizomenon population, initially concentrated in the surface layers, spreads down into the anoxic waters through the thermocline later in the summer. Ceratium becomes quantitatively dominant later in the summer, with large numbers of Aphanizomenon concurrently present. No nitrogen fixation has been detected here, and ammonia is the chief nitrogen source used.

NITROGEN UTILIZATION IN CIRCLE HOT SPRINGS

Blue-green algae are characteristic components of the populations in hot springs. Only 50 miles south of the Arctic Circle, Circle Hot Springs represents a unique environment. The dominant algae here are

ENVIRONMENTAL REQUIREMENTS OF BLUE-GREEN ALGAE

Mastigocladus laminosus and Phormidium sp. The springs emerge from a small opening in the rock and descend rapidly, with one major pool near the source. The rest of the water drains down, and is used to supply a swimming pool and also to heat the resort buildings. Nitrogen uptake measurements show that nitrogen fixation is the quantitatively most important nitrogen uptake, although low rates of uptake of nitrate and ammonia were detected. These data are shown in Table 5. The water temperature at the source is about 55°, and the optimal growth of the Mastigocladus in the laboratory was at 45°C, with no growth below 35°C. Phormidium grows at room temperature and seems to tolerate a broader range of temperatures.

Table 5

Uptake rates of nitrogen sources by particulate fraction
in Arctic Circle Hot Springs water, Alaska - June 1963

	Atom % Excess	$\mu\text{g N}$ $\text{liter}^{-1}\text{hr}^{-1}$	
Nitrate - ^{15}N (90%)	.096	.04	Total nitrogen concentration in particulate fraction, 885 $\mu\text{g/liter}$
Ammonia - ^{15}N (98%)	.280	.10	
Nitrogen gas - $^{15}\text{N}_2$ (95%)	2.552	.99	
Algae present: <u>Mastigocladus laminosus</u> <u>Phormidium</u> sp.			
Incubated "in situ" in hot spring pool at $\sim 45^\circ\text{C}$.			

DISCUSSION

The significant questions which have been asked in this work are:

- What is the nitrogen nutrition of naturally occurring blooms of blue-green algae, and especially, how important is nitrogen fixation?
- Under what conditions do major nitrogen-fixing blooms develop?
- How do these populations fit into the seasonal patterns of algae production?

Aspects of Nitrogen Nutrition of Some Blue-Green Algae

Nitrogen fixation is apparently highly significant under some conditions. In general, shallow depth, high organic content, high temperatures, and high insolation appear to be prerequisites. The availability and presence of combined nitrogen sources in the water does not appear to prevent nitrogen-fixing blooms from developing. Shallow warm waters in the Arctic and Subarctic apparently provide suitable conditions for such blooms in the summer. In Table 6, I have summarized some rates of nitrogen fixation measured in lakes ranging from Pennsylvania to the most northern tip of the United States, at Point Barrow, Alaska. In Smith Lake, the total nitrogen fixed during a season is approximately $500 \mu\text{g liter}^{-1}$, which represents a significant proportion of the budget.

The role of bacteria in algal cycle is unfortunately little known, although a start is evident in the paper on microbiotic cycles by Silvey and Roach (1964). Whereas in Sanctuary Lake there is important inflow of sewage-laden water with varying nutrient concentrations, which seem to influence the algal periodicity, in Smith Lake internal factors appear to be more important. The rapid release rate of ammonia from algal populations suggests that the development of bacterial populations in response to algal cycles is a common feature.

Ammonia, the energetically economical form of nitrogen, is assimilated readily by blue-green algae, and *Anabaena* in particular can grow rapidly on ammonia at a low concentration but with a high supply rate, and under these conditions supplement its nitrogen income by nitrogen fixation.

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Table 6

A summary of some nitrogen fixation rates

Sanctuary Lake, Pennsylvania	<u>Anabaena circinalis</u> <u>Anabaena spiroides</u>	47 $\mu\text{g liter}^{-1}\text{day}^{-1}$
Smith Lake, Alaska, 1963	<u>Anabaena flos-aquae</u>	1 $\mu\text{g liter}^{-1}\text{hr}^{-1}$
Smith Lake, Alaska, 1964	<u>Anabaena flos-aquae</u>	3 $\mu\text{g liter}^{-1}\text{hr}^{-1}$
Circle Hot Springs	<u>Mastigocladus laminosus</u>	1 $\mu\text{g liter}^{-1}\text{hr}^{-1}$
Paul's Pond, Pt. Barrow (from Dugdale and Toetz, 1961)	<u>Nostoc sp.</u>	3.34 $\mu\text{g liter}^{-1}\text{day}^{-1}$
Little Kitoi Lake (from Dugdale and Guillard, 1966)	<u>Anabaena sp.</u>	.04 $\mu\text{g liter}^{-1}\text{day}^{-1}$

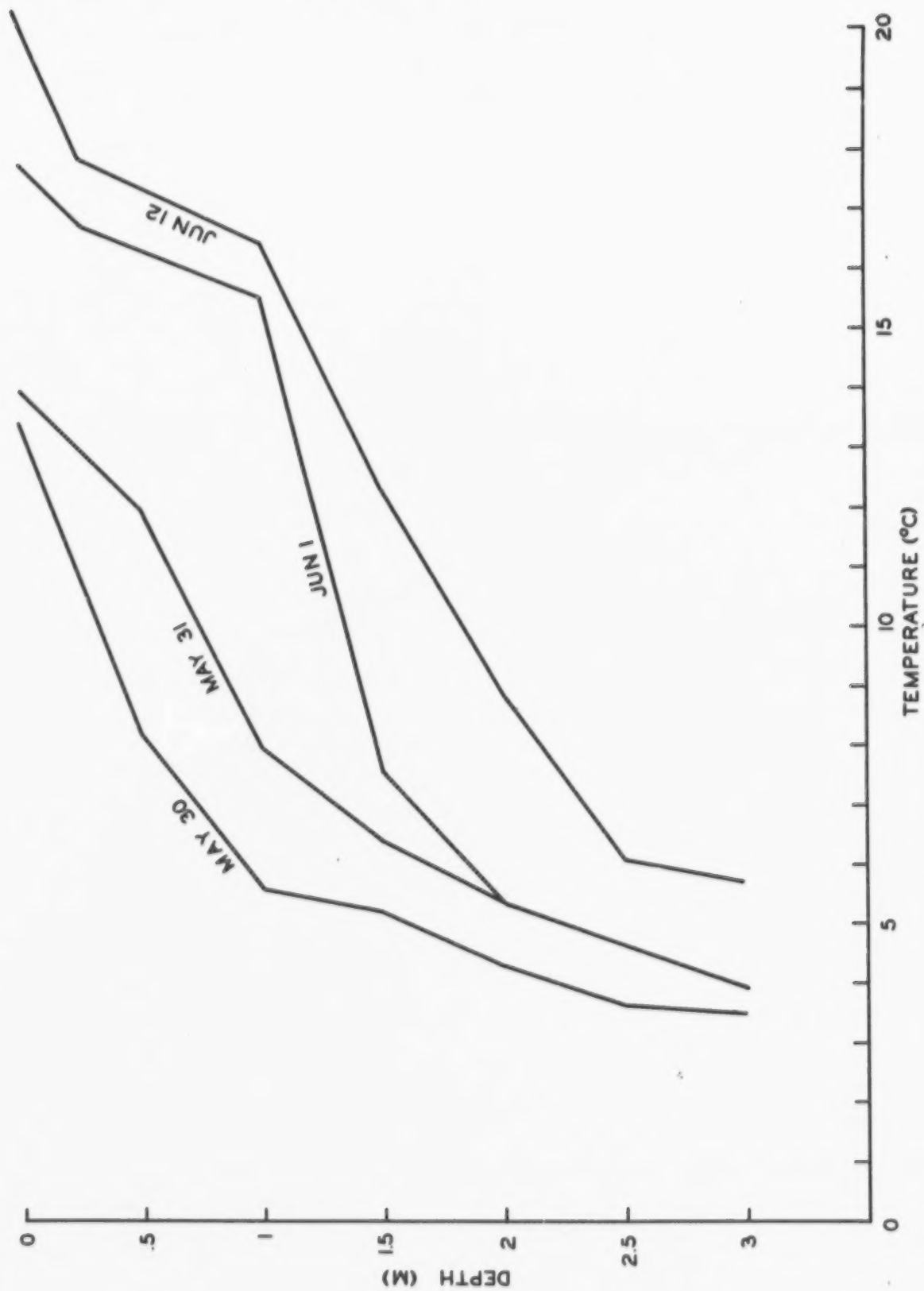


Figure 1. The depth-distribution of temperature during the *Anabaena* bloom, Smith Lake, 1964.

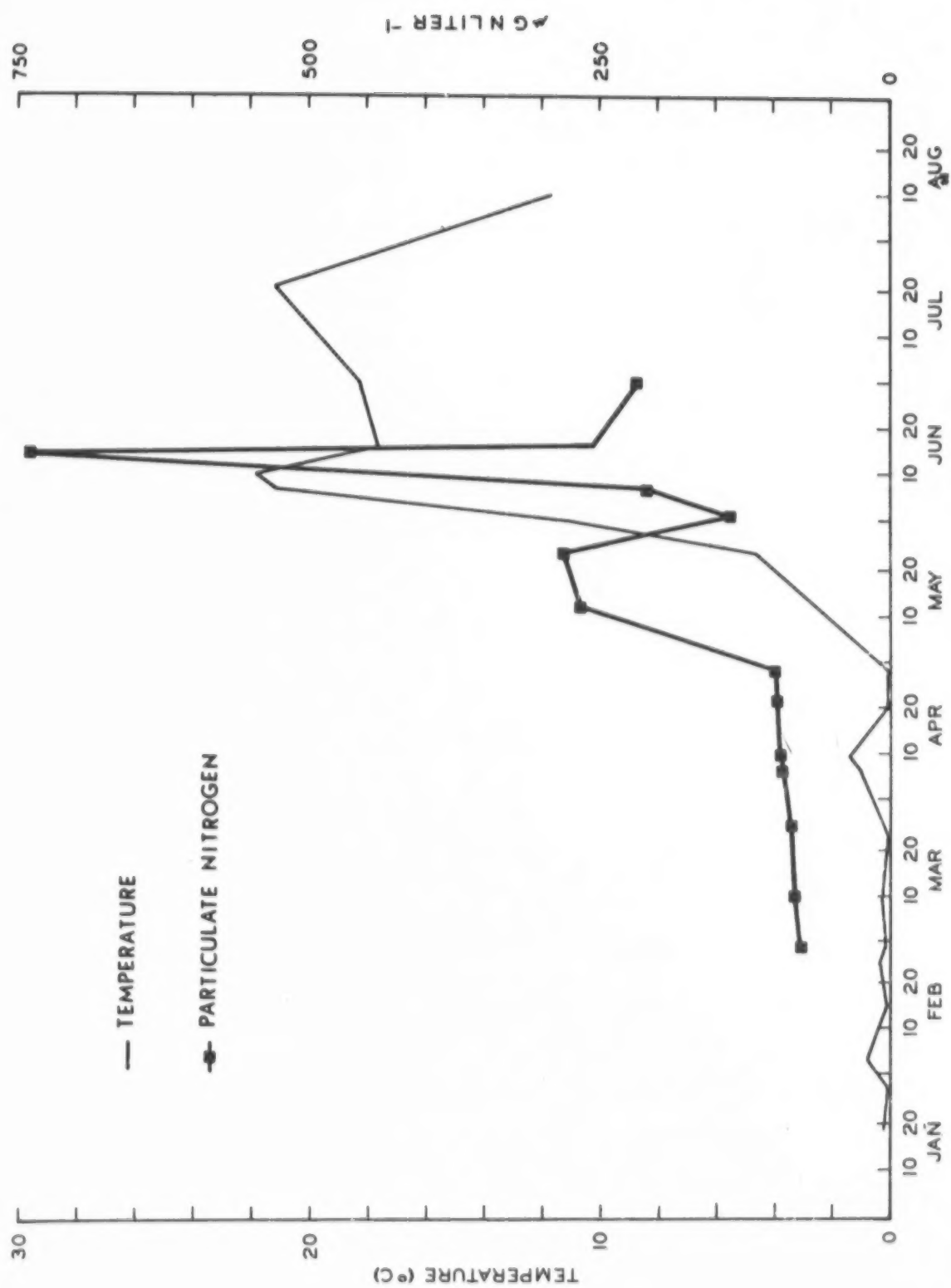


Figure 2. The relationship between surface temperature and particulate nitrogen in Smith Lake, 1966.

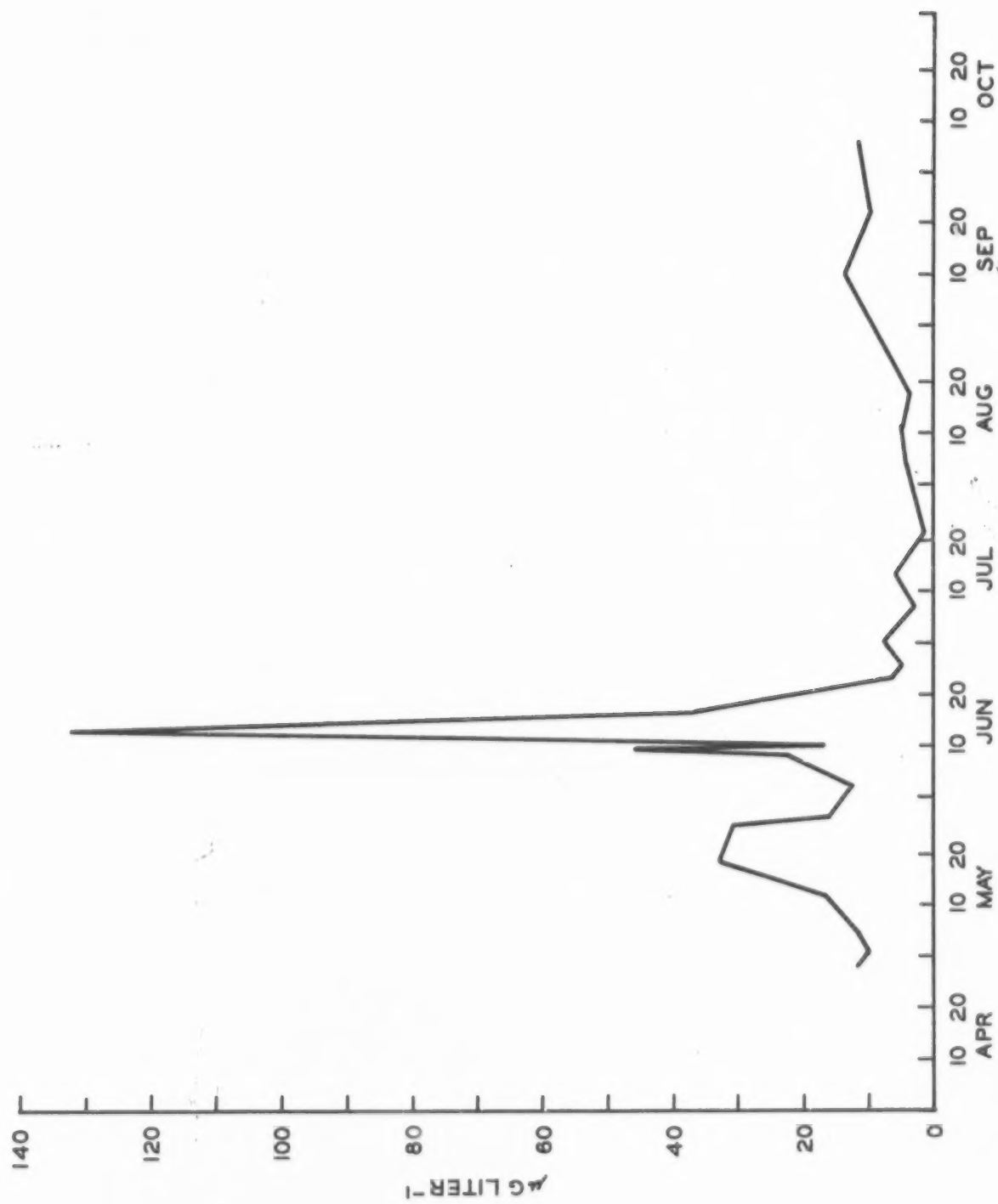


Figure 3. The seasonal distribution of chlorophyll *a* concentration, Smith Lake, 1964.

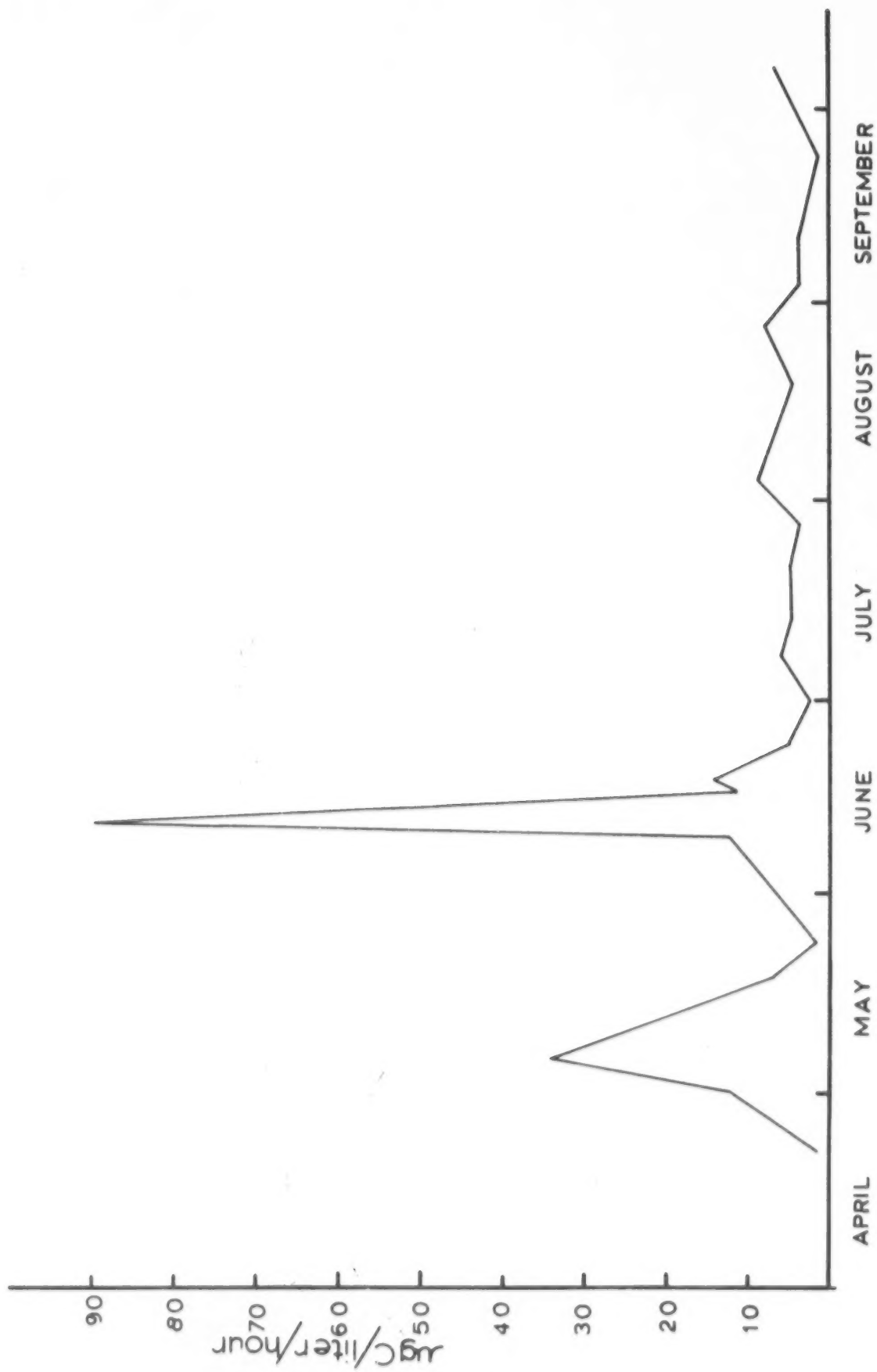


Figure 4. The seasonal distribution of primary productivity, Smith Lake, 1964.

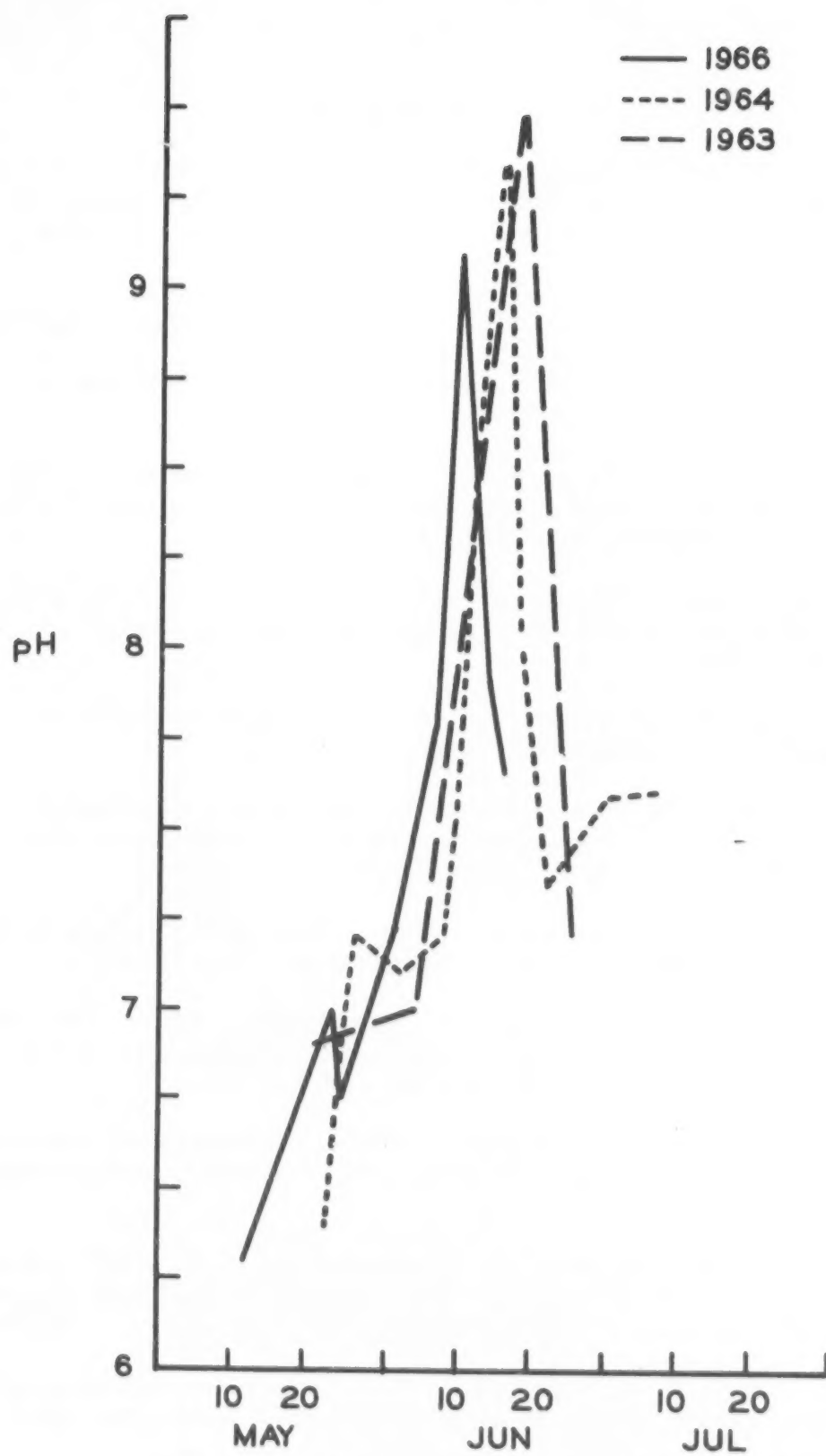


Figure 5. Some representative pH curves for Smith Lake surface water.

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ENVIRONMENTAL REQUIREMENTS
OF THERMOPHILIC BLUE-GREEN ALGAE

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INTRODUCTION

This discussion will relegate itself primarily to that habitat and those organisms above 45°C. This is not a completely arbitrary breaking point between the mesophilic and the obligate thermophilic algae. Most of the latter find optimal conditions above 45°C and will generally not grow at temperatures much below 30°C. With one confirmed exception, the eucaryotic algae reach their upper limit at about 45-47°C. Only the enigmatic Cyanidium caldarium breaks beyond this barrier well into the thermal range. Anacystis nidulans, so widely used as an experimental organism, has an upper growth limit of about 45°C and an optimum of 41°C (Kratz and Myers, 1955), falling somewhat short of a true thermal range. Probably, a majority of the blue-green algae have tendencies in the direction of thermophily. R. Y. Stanier (personal comm.) has informed me that holding the temperature at 35°C is a simple enrichment method for obtaining a wide variety of blue-green algae from crude collections containing many other types of algae. Subsequently, I have had similar success with a number of non-thermal collections at 37°C. Naturally, a considerable number of blue-green species will not fall into this category. Nor would this method be well suited for collections from the non-thermal portion of a hot spring drainway, since in this environment there are a number of green algae and diatoms adapted to temperatures of up to 45°C. It is not unusual for micro-algae to grow at maximal rates at temperatures significantly higher than they would normally encounter in nature (e.g. Braarud, 1961), but blue-green algae seem to lie 5 to 15 degrees higher up the scale than the others.

The non-planktonic blue-green algae demonstrate their greatest development in hot springs, particularly in the formation of macroscopic mats of a wide variety of textures, colors, and thicknesses (e.g. Schwabe, 1962a, 1964). Above the 45°C border the blue-green algae are essentially alone in their nutritional mode with only an unknown quantity of heterotrophs, chemolithotrophs, and a few photosynthetic bacteria as unlikely competitors for the same niches. Under the upper photosynthetic layer of the gelatinous or leathery mats one usually finds several multicolored layers which either lack photosynthetic

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capacity or consist primarily of dead material. The accumulation of this material is a strange phenomenon. In a temperate environment this material would normally be mineralized by heterotrophs more in pace with primary production. Heterotrophic, thermophilic eubacteria can be isolated from any temperature at which blue-green algal mats occur, but they do not seem to be abundant. When such mats reach an encumbering thickness or age, peeling or washouts by catastrophic rains result in transport to a non-thermal environment where breakdown may occur rapidly.

Perhaps one of the most important reasons for the great mat development in thermal waters is the absence of grazers above about 45 to 47°C. For example, in Yellowstone Park, Ephydrid larvae erode the algal mat significantly in some alkaline springs below 43°C. In Hunter's Hot Springs, Oregon, a very abundant ostracod of the genus Potamocypris grazes on the surface of the Phormidium and Pleurocapsa mat between 47° and 30°C.

The most obvious environmental requirement of a thermophilic blue-green alga is high temperature, but many have probably adapted to some extraordinary water chemistries as well. Certainly, not all hot spring waters are potable, nor are they balneal "cure-alls". Some of the thermal waters are certainly highly eutrophic, containing an ample or excessive supply of macro- and micro-nutrients. They exhibit relatively high salinities and may contain various metals in concentrations so high that they should be labelled poisonous. It is possible, however, that higher concentrations of certain metals may impart greater thermal stability to some organisms (e.g. Ljunger, 1963; Militzer and Burns, 1954).

A general adaptation to high light intensity should also be expected in most thermophilic algae. The usual shallow outflow from a hot spring results in exposures to extremely high light intensities, with little or no refuge.

Much of the information relating to thermophily has come from the thermophilic bacteria. In addition, so much must be inferred from work with mesophilic Cyanophyta. True thermophilic blue-greens have not been generally available from stock collections, and few research institutions are located within commuting distance of hot springs. Also, the inconvenience of maintaining high temperature equipment and the general belief that thermophilic algae would be difficult to culture have kept their use in the laboratory to a minimum.

The gross chemistry of quite a number of hot springs is known, and there are some published data on the responses of thermophilic blue-greens under laboratory conditions. However, little synthetic

ecology has emerged from these two sets of information. Only now has some experimental work begun on the organisms within their native environment.

GEOGRAPHIC AND HISTORICAL FEATURES

Although isolated warm or hot spring groups exist in many parts of the world, there are only a few areas of extensive thermal activity. The concentrated areas are in the western United States (Yellowstone Park), Iceland, New Zealand (Rotorua), and Japan. Hot springs are scattered through central and southern Europe, Asia, the East Indies, Melanesia, Africa, and South America, but they are missing entirely from several large land areas. Most are found in areas of Quaternary or current vulcanism, although many groups of springs are associated entirely with diastrophism and lie near major faults. Of the four main areas, only Iceland lies above 60° of latitude.

Although aquatic thermal habitats of similar types are widely isolated geographically, there is a great similarity of the flora throughout the world. However, lacking physiological, biochemical, or genetical evidence of similarity, it becomes difficult to state that two similar blue-green algae are similar in anything besides morphology. Schwabe (1962b) brings up the difficult problem of species characterization and meaning in the Cyanophyta. It is obvious that there is a considerable amount of endemism among the thermal blue-green algae, even with respect to strictly morphologically defined species.

The major thermal areas of the earth have apparently been active continuously since some portion of the Pleistocene. Although little is known about dispersal mechanisms in the thermophilic Cyanophyta and survival capacities during transport, one might suppose that close to a million years or more would be sufficient time for all species to have been inoculated several times into all of their potential habitats. This is fallacious. Many hot spring areas have developed in quite recent times, and it is probably safer to assume that many of the blue-green algae disseminate in manners similar to those of the eucaryotic microalgae which they resemble in cell size and general morphologic pattern. In some of the freshwater Chlorophyta, time and distance are demonstrable factors in distribution patterns. The absence of some species in many thermal waters may be due to the lack of a suitable habitat of lasting duration rather than the lack of inoculation opportunity, but the latter is still a real possibility.

Barghoorn and Tyler (1965) and others have recently found good micro-fossils of blue-green algal-like filaments and cells in Precambrian

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strata. There is evidence indicating that some of this material was in an aquatic paleoenvironment with a high silica content, similar to that of present hot springs. It becomes attractive and reasonable to believe that some, at least, of the present thermophilic Cyanophyta are directly descended from Precambrian thermophiles and are not more recent invaders of the thermal environment. Such a hypothesis assumes that there was no discontinuity in the aquatic thermal activity during the interim.

TEMPERATURE CHARACTERISTICS AND LIMITATIONS

Constancy is one of the most characteristic features of thermal waters. Many spring sources, ranging from 40°C to boiling, vary little in temperature or dissolved mineral composition even over periods of many years. Yet, it is not safe to assume that this is the case for every spring encountered. Pools of geyser craters and superheated springs often vary considerably in temperature in the course of a few hours, and, in the case of geysers, the changes in overflow volume naturally result in temperature changes along the drainway. Some streams from geyser pools may flow only intermittently during and preceding an eruption. Many thermophilic or "semi-thermophilic" algae form aerial mats and depend entirely on frequent wetting by geyser spray (Fig. 1).

Little is known about the resilience of thermophilic blue-green algae subjected to temperature changes. Peary and Castenholz (1964) showed that several strains of Synechococcus lividus (one growing as high as 70°C) are able to tolerate abrupt changes in temperature within their growth range or from room temperature to any point in that range without apparent damage. Temperature shocks to above the long-term lethal limit are also tolerated provided that these temperatures are not held for long (Peary, 1964). Resilience of this type appears to be characteristic of obligate thermophilic bacteria as well, but rapid shifts up the thermal scale are usually fatal to facultative thermophilic bacteria (those that grow well at 37° and 55°C--Bausum et al., 1961).

A lack of resilience was noted by Marrè and Servettaz (cited in Marrè, 1962) in Aphanocapsa thermalis. This species was very sensitive to changes from its temperature of acclimation, whatever that happened to be. Lowering the temperature several degrees depressed both respiration and photosynthesis, but particularly the latter. Raising the temperature only a few degrees caused a decline in photosynthesis which was irreversible, followed by a similar decline in respiration. The gap between the acclimation temperature and the inactivating high

temperature became less the higher the temperature of acclimation. In Synechococcus lividus (collected at 53°C), Peary (1964) found no such indications of acclimation or deadaptation. The clone culture was grown for over one year at 50°C before a sub-culture was established and maintained simultaneously for eight additional months at 30°C. The normal growth rate at 50°C was 8 doublings per day, and at 30°C it was between 1 and 2 doublings per day. The optimum was at 45°C at over 9 doublings. At the end of the entire period the growth rates for both 30°- and 50°-maintained cultures were both normal throughout the total range from 30° to 55°C. Exponential growth commenced at 45° and 50°C, with essentially no lag with inoculum from 30° or 50°C stock. Allen (1959) and Löwenstein (1903), on the other hand, found that the maintenance of a thermophilic alga below its optimal growth temperature for a long period resulted in a deadaptive lowering of the optimal growth temperature and the upper tolerance limit. The organisms were Cyanidium caldarium (non-Cyanophyta) and Mastigocladus laminosus, respectively. Little is known of genetic adaptation in the blue-green algae. Mutability has been demonstrated only a few times (Singh and Singh, 1964a,b; Van Baalen, 1965).

The upper temperature limit for the growth of blue-green algae is still unresolved. Copeland (1936) made observations of blue-green algae in hot springs of Yellowstone Park at temperatures above 75° and up to 85°C. Others (cited in Peary, 1964) have indicated the presence of blue-green algae above even 90°C. Recently, however, there has been no incontestable evidence of the growth of chlorophyll-containing organisms at constant temperatures above 74-75°C. Kempner (1963), looking at upper temperature limits of life in a few adjacent hot springs in Yellowstone Park, concluded, on the basis of P³² incorporation into nucleic acids, that 73°C was about the upper temperature limit for growth. This corresponds well with observed demarcations of Synechococcus mats in hot springs of the same area in Yellowstone Park. In a recent two-week visit to Yellowstone I explored a number of the hot spring and geyser basins. Some of this was with Thomas D. Brock, of Indiana University, who is currently working on the productivity and general ecology of some thermal springs there. In all of the alkaline and neutral springs investigated, 73-74°C seemed to be the absolute upper limit for a visible cover of Synechococcus (Fig. 2). Some of the areas explored were those mentioned by Copeland as containing one or more of the five blue-green species whose upper limits were above 80°C. It is indeed true that there are non-photosynthetic organisms that live in springs of the Yellowstone at temperatures of over 80°C. Brock (personal comm.) found no chlorophyll in a pink-colored filamentous organism growing up to about 88°C and forming luxuriant trailing tufts at this temperature. It is possible that three of Copeland's high temperature species of Phormidium and Oscillatoria should be

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referred to the non-photosynthetic "flexibacteria" or apochlorotic blue-green algae. Neither Setchell (1903) nor Nash (1938) reported blue-green algae above temperatures in the mid-70s in Yellowstone springs, which agrees with our findings. The most intriguing recent report of a higher limit is that by Anagnostidis (1961) of Synechococcus at 81°C in a salt spring of Greece. A demonstration of growth is, of course, required to confirm any of the above field observations.

The 74-75°C upper limit for blue-green algae does not extend to thermal waters of all chemical types or to all geographical locations. In Iceland Schwabe (1936) found blue-green algae at no higher than 60.2°C. Brock and Brock (1966) and Jon Sperling (personal comm.) have indicated that the highest blue-green habitat was about 60° and 63°C, respectively. These temperatures reflect the approximate upper growth limit of Mastigocladus laminosus in Iceland; this is also the upper limit for this cosmopolitan species elsewhere (Schwabe, 1960). Iceland definitely has an impoverished thermal flora. Petersen (1923), who studied all the freshwater Cyanophyta, identified about 39 thermal species. This is less than one-half the usual number found in hot spring groups of lower latitudes. Schwabe (1936) reduces this number to six definite thermal species typical of the alkaline springs. Species of Synechococcus, which generally contribute the upper temperature forms in North America, Japan, and Greece, appear to be entirely missing from Iceland. Either this group has not been inoculated into Iceland yet, or the environmental conditions are not suitable for it. Although the volcanic activity has apparently been continuous for somewhat less than a million years, an ice cap covered the island until Recent times. The thermal springs present during the ice period may have been severely handicapped and variable. At present, the chemical characteristics of many North American springs with Synechococcus are essentially duplicated in many Icelandic springs. The winter light condition may be the most detrimental facet of the environment.

In the New Zealand hot springs, an upper limit for blue-green algae of 61°C has been cited by Nash (1938). Again, a wide variety of thermal and chemical conditions exist, but again Mastigocladus laminosus forms the upper boundary (along with Phormidium sp.). Some species of Synechococcus are present in the springs. Since North Island lies at about 40° latitude, it shares with Iceland only the properties of being insular and at a great distance from other thermal areas.

Besides the differences in upper temperature limits between Yellowstone and Iceland, Brock and Brock (1966) report that standing

crops of blue-green algae also reach maxima at different temperatures in the two locations. In an alkaline spring in Yellowstone, maxima of chlorophyll, protein, and RNA occurred at about 54°C, whereas, in an Icelandic spring, the same components of standing crop peaked at about 48°C. The difference could easily be attributed to the species composition, which might in turn reflect chemical differences or geography, or both. Brock (personal comm.) has reported that primary productivity in the Yellowstone spring, as measured by C^{14} assimilation per unit area, peaks in the area of maximum standing crop to somewhat lower temperatures. A standing crop and productivity maximum in the mid-50s was apparent in the Drakesbad Hot Springs of Mt. Lassen National Park, California (Lenn, 1966).

From the temperature of maximum abundance to the upper limit of photosynthesizing blue-greens, standing crop and the number of species declines steeply (e.g. Brock and Brock, 1966; Vouk, 1950). This is quite apparent visually in North American hot springs, at least above 60-65°C. In Hunter's Hot Springs, Oregon, the one or two strains of Synechococcus of the highest temperature type form only a thin mat and grow at relatively poor rates in culture (Peary and Castenholz, 1964). Without aeration and additional CO_2 the medium may have been a limiting factor at 65° and 70°C, however. In the case of the Oregon spring, Synechococcus formed a continuum from about 74° to 54°C, at which point Oscillatoria terebriformis entered the spectrum and became predominant. A minimum of four temperature strains of the Synechococcus may form an overlapping series in the continuum.

Marrè (1962), in a recent review of temperature and algae, alludes to the lower growth rates of thermophilic algae. In fact, little to nothing is known of their growth rates in nature. In culture, however, the growth rates of many are considerably higher than the norm for well-known mesophilic and psychrophilic algae. Synechococcus lividus (Clone 53), in my laboratory, can reach 10 doublings per day at 45°C, and Oscillatoria terebriformis can approach 5 doublings at its optimum of 50°C. Dyer and Gafford (1961) have also reported 9 doublings per day for Synechococcus lividus from Yellowstone.

A property generally ascribed to thermophilic algae, in particular, is the ability to grow or photosynthesize at maximal rates up to a temperature close to the lethal upper limit (e.g. Marrè, 1962; Prat, 1956). In nature this may result in the formation of a prominent uni-algal mat which is neatly delimited at its upper end from the neighboring species up the thermal gradient. Sudden increases in temperature down the range should result in easily observed changes in the visible pattern of such mats. The sharp banding and fine lines of demarcation are some of the most striking features of hot springs the world over

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(Fig. 3). The observed temperature range of a particular species in a hot spring usually corresponds to the growth temperature range in the laboratory only with respect to the upper limit. The sub-optimal growth temperature range in the laboratory is masked in the field by the competitive predominance of another species which finds its optimum within that range. In Hunter's Hot Springs Oscillatoria terebriformis successfully competes with at least one of the Synechococcus strains in the optimum temperature range of both (Peary and Castenholz, 1964).

A maximal growth rate just below the upper temperature limit does not appear for all strains of Synechococcus. The optimum was often 10-12 degrees below the limit. Holton (1962) found a similar case in Mastigocladus laminosus when the culture was aerated with line air. The broad growth optimum was narrowed considerably and moved towards the upper temperature limit with 1% CO₂ in air. However, the upper limit was depressed about 5 degrees to 55°C under the latter conditions. Aeration might also change the pattern in the temperature response curves of Synechococcus (Peary and Castenholz, 1964). Oscillatoria terebriformis, however, maintains its growth peak to only 1 or 2 degrees below its upper limit of 54°C, with or without aeration.

The lower temperature limit of growth in obligate thermophilic blue-green algae differs greatly with species; it is often well within the temperate range. The loss of growth potential at very moderate temperatures may also be accompanied by lethal lesions (Forrest, et al., 1957). A very high temperature strain of Synechococcus (Clone 75) finds its lower growth limit somewhere between 50° and 55°C. Other strains ceased growth between 45° and 50° (Clones 66 and 71), between 35° and 40° (Clones 55 and 60), and at some point below 30°C (Clones 45, 48, and 53) (Peary and Castenholz, 1964). Oscillatoria terebriformis, from the same Oregon hot spring, will not grow under normal circumstances below about 30°C (Castenholz, unpubl.). I have recently investigated the effect of low temperatures on this species. It is one of the most abundant organisms in Hunter's Hot Springs, forming dense mats of entangled and tufted trichomes from about 54° to 47-45°C in the thermal gradient (Fig. 3 and 4). Although peaking at about 50°C, the general growth optimum for this species in culture fits the distribution of the mat in nature, even at the lower end. Below approximately 43°C in culture growth declines to the minimum at 30°C. This species exhibits rapid oscillating movements which result in a spectacular aggregation phenomenon (Castenholz, 1967). The rate of this form of motility is saturated only above 37°C, but it occurs even as low as 12°C in the light and at a respectable rate between 20° and 30°C. Below about 27°C, however, the motility can be accounted for almost entirely by energy supplied through photo-

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phosphorylation. Presumably, the enzymes associated with respiration or oxidative phosphorylation are most inactivated at this temperature and below. Below 27°C, the Oscillatoria will hardly aggregate in the dark, while at optimal temperatures dark aggregation takes place with almost the same rapidity as in the light. Prat (1954, 1956) demonstrated that photosynthesis continued at least 20 degrees below the lower growth limit of Mastigocladus laminosus (28-30°C) and that respiration occurred for about 15 degrees below. However, growth, photosynthesis, and respiration all reached maximal rates at about 40° C in this strain.

Tentatively, I would consider the aggregative behavior of O. terebriformis as an adaptive feature which enables the trichomes to hold together as a "mat" within the optimal temperature range in flowing water (Fig. 4). As may be seen in the photograph, trichomes are not embedded in a gelatinous mat but are free or roped together. Vigorous agitation causes almost complete dispersal of the natural mat. The great majority of blue-green algae in thermal spring drainways hold their position in the flow by the formation of a gelatinous matrix or else by embedding or multiplying within the matrix formed by other organisms.

Being washed downstream in a hot spring to moderate or cold temperatures may be lethal to most of the obligate thermophiles, although the fate of thermophilic algae at low temperatures is largely unknown even in the laboratory. Oscillatoria terebriformis dies when exposed to lower than growth temperatures for extended periods (Castenholz, unpubl.). The deterioration preceding death is more rapid the lower the temperature and the higher the light intensity. For example, at the end of 6 hours at 14°C and a light intensity of about 2,000 ft.-c. or 12-24 hours at 450 ft.-c. complete loss of viability occurred, as evidenced first by the loss of motility when raised to normal temperatures and then by the leakage of the phycobilin pigments into the medium. Increasing the temperature or lowering the light intensity prolonged the period of viability, so that at 23°C and 450 ft.-c. death occurred only after 30 days. In darkness, at 45°C, O. terebriformis dies in from 24-48 hours, but at 25°C it will remain in good condition for over a week. In the same period there was poor survival in darkness at 11°C and even less at 3°C. Synechococcus lividus (Clone 53), on the other hand, is relatively insensitive to low temperature storage in light or darkness. A high temperature strain (Clone 75), which grew from 55°C to over 70°C, evidenced some survival after 10 days at 30°C in the light (Peary, 1964).

The survival of thermophilic algae outside of their milieu is a problem continuously encountered but about which little is known. Few of the thermophiles have morphological resting spore stages, but many can probably tolerate desiccation, nevertheless. This may be true in

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particular for those cells drying within the "gel" of a mat. Synechococcus lividus (Clone 53) will not survive drying much above room temperature, and the survival rate is apparently very low even at 25°C after drying 24 hours over anhydrous calcium sulfate. Oscillatoria terebriformis, however, will not tolerate drying under any conditions, even momentarily (Castenholz, unpubl.).

Tolerance to freezing in blue-green and green algae was recently investigated by Holm-Hansen (1963). He found evidence of adaptation in that the various algae from the Antarctic were able to survive freezing while all but one green alga from Wisconsin was killed by either slow or rapid freezing. For most of those algae that survived freezing, slow freezing was less damaging than rapid. On the other hand, all the strains of thermophilic Synechococcus in my laboratory were able to tolerate slow freezing (5-10 minutes) and retain a high survival percentage after storage at -20°C for a period of at least 10 months. Oscillatoria terebriformis will not survive freezing, either rapid or slow.

In the middle or temperate latitudes the winter survival of thermophilic algae should require no special mechanism, since there is adequate light for the maintenance of growth. The thermal gradient contracts, and productivity may be reduced considerably, but no difficulties in species survival should arise. In Hunter's Hot Springs in winter there is little change in appearance from summer except for a contraction of the species bands toward the source. Also, there is little change in the relative species abundance (Peary, 1964). In the extensive springs of Iceland, where the sun may appear for only three hours or less at a very low elevation in mid-winter, the algal cover apparently breaks down and does not recover fully until late spring or summer (Schwabe, 1936; Brock, personal comm.). Some forms may survive without growth in tepid or cold niches in the drainways. Others may survive dried or frozen along the edges of the springs. Heterotrophy, rare in blue-green algae, is an unlikely winter survival mechanism. Forms such as Oscillatoria terebriformis theoretically should not be able to survive an Icelandic winter. It is a wonder how this species has been disseminated any distance anywhere. It is reported from thermal habitats of western North America, southern Europe, and Japan, but not from Iceland. The species name is also used for a non-thermal Oscillatoria from a much greater geographical range. Certainly several taxa are represented. They may all fit within the dimensions of the original description, but temperature ranges, color, and growth habits do not agree.

RESPONSES TO LIGHT INTENSITY

As mentioned earlier, many or most of the hot spring environments are subjected seasonally to very high light intensities because of locations at high elevations, in treeless areas, or in areas where the surrounding trees have been destroyed by the springs and fumaroles. The Yellowstone hot spring basins lie between 6,000 and 8,000 ft. elevation. Hunter's Hot Springs of south central Oregon occur in the prairie at about 4800 ft. elevation. In Yellowstone a maximum of about 0.6 g cal/cm² per min. of radiation in the visible range is received at the surface in summer; a maximum of about 0.2 g cal/cm² per min. is received on a clear winter day (see Gates, 1961, 1962). Since most of the thermal pools are very clear and the stream outflows very shallow, little light extinction would occur. One would expect a large number of sun-adapted forms little affected by the highest summer intensities and exhibiting rather high growth saturation and compensation intensities. Few laboratory studies have been made.

One of the most striking visual aspects of hot springs is the multicolored algal pattern in which oranges and yellows are conspicuous, particularly in summer. Sargent (1934) was one of the first to investigate the color change in blue-green algae, using fairly modern methods. He found that a high intensity of white light or of any broad color band caused a change from dark blue-green to yellow in the non-thermal Gloeocapsa montana, and that lowering the intensity reversed this process. The color change could be explained solely by the reduction in cell chlorophyll content which was inversely proportional to light intensity. Brock (personal comm.) has controlled the greening and yellowing of high-temperature Synechococcus populations in situ through the placement and removal of shades. With O. terebriformis, using light intensities from 200 to 4,000 ft.-c., I found a drop in chlorophyll content per unit dry weight to less than 20% of the highest value at 200 ft.-c. (coolwhite fluorescent). Total carotenoid pigments dropped less than 50% in the same range of intensities. Thus, the carotenoids become relatively more important with increasing light intensity. The typical low-light or aggregated Oscillatoria is a dark reddish-brown; the dominant color imparted by phycoerythrin. The trichomes change to a pale ochre color with high light; the phycoerythrin is reduced in parallel with chlorophyll (Castenholz, unpubl.). Similar results were obtained with Anacystis nidulans (Myers and Kratz, 1955). Thus, pigment adjustments occur in response to light intensity. Preliminary evidence suggests that in O. terebriformis the adjustment of pigment ratios is a necessary prerequisite to the unrestrained maximal mass increase. The photo-protective role of carotenoids has already been well established, particularly in photosynthetic bacteria (e.g.

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Sistrom et al., 1956). Complementary chromatic adaptation and the inverse type are also confirmed in the recent literature of the blue-green algae (Fujita and Hattori, 1960; Jones and Myers, 1965).

It is difficult to discuss the effect of light intensity independently from temperature, since the two factors are so completely entangled (e.g. Sorokin and Krauss, 1962). For example, at 45°C, Oscillatoria terebriformis showed growth saturation at about 1,200 ft.-c., while at 31°C saturation occurred at about 350 ft.-c. and the growth rate was cut to about 20% of the 45°C value (Castenholz, unpubl.). High light (3,000 to over 4,000 ft.-c.) is not inhibitory to the growth of this organism at 45°C, but at 31°C, 1,500 ft.-c. depressed the rate significantly. The aggregated mat in nature at high summer intensities showed an average carotenoid to chlorophyll ratio equivalent to that which would be expected with a constant exposure to about 1,000-1,500 ft.-c. in the laboratory (dispersed trichomes). The nature of the mat, with possibly moving and interweaving trichomes, may prevent individual trichomes from being exposed to a constant daytime high light intensity or to the low intensity of continual submergence in the mat. The mat as a unit might be operating at less than a saturating light intensity, particularly in a light-dark regime.

For Synechococcus lividus (Yellowstone--50°C), Sheridan (1966) also found a high light intensity for photosynthetic saturation at 45°C (600-900 ft.-c.). Dyer and Gafford (1961) showed lack of saturation at 400 ft.-c. in Synechococcus lividus from 45° to 55°C; 1,500 ft.-c. was the next highest intensity used. Fukuda (1958) showed that photosynthetic saturation in the acidophilic thermophile, Cyanidium caldarium, fell between about 1,000 and 10,000 ft.-c. when CO₂ was not limiting. The highest intensity (10,000 ft.-c.) was not inhibitory. In acid thermal creeks in Yellowstone Park (e.g. Roaring Mt. creeks--pH 2-3) it is obvious that above 50-55°C, high natural light intensity becomes quite limiting to growth near the upper temperature limit (ca. 60°C). In this thermal range visible covers of Cyanidium appeared only in shaded niches.

In thermal springs there is some evidence to indicate that photosynthetic incorporation of C¹⁴ into the algal mat fluctuates in parallel with natural sun-plus-sky radiation even during the summer period of generally high energies (Lenn, 1966). Stockner (1967) has shown that the growth of Schizothrix calcicola (= Synechococcus sp.) in Ohenapecosh Hot Springs (Mt. Rainier National Park) paralleled very closely the average sun-plus-sky radiation flux over the course of a year. A difficulty encountered in measuring the native rates of photosynthesis or growth relative to light intensity is the thickness of the algal mat. The active chlorophyll-bearing cells occur only in the

upper few millimeters, but the density of this top layer may be great enough to cause significant reductions in the light intensity received by the lower cells. Thus, light intensity reaching the surface cells may seldom be limiting while a large portion of the buried cells may be receiving light well below saturation levels. C^{14} incorporation by sections of the mat may represent the average responses to a complete gradient of light intensities.

DISSOLVED SOLIDS OF THERMAL WATERS AND NUTRITIONAL ASPECTS

The total dissolved solid content of hot springs varies considerably from area to area, but there are few with less than 500 mg/l TDS and more than 2,500 mg/l. An average value might lie somewhere above 1,000 mg/l. These values are well above those of the normal stream and lake systems which receive most of their water from relatively recent precipitation. Practically all of the 3,000 or more hot springs in Yellowstone Park have salinities between 1,000 and 3,000 mg/l (Allen and Day, 1935). Extremes are of interest. Medicine Hot Springs, Montana, has less than 250 mg/l TDS and a hot spring in Iceland issues seawater enriched to over 47,000 mg/l but with a pH of 5. The latter appears to be devoid of algal growth (Brock, personal comm.). Other very saline hot springs are known in the salt dome regions of northern Utah, and springs with salt concentrations of over 15,000 mg/l in Greece have an abundant blue-green algal flora (Anagnostidis, 1961). Halophilic blue-green algae are well known as some of the predominant organisms in inland saline lakes.

The mineral content of many or most hot springs probably remains very constant. However, there are exceptions in which the concentration at the source may vary erratically or seasonally, often under the influence of rainfall in the watershed. The ratios of certain elements may also change considerably within a short period (Uzumasa, 1965). Most hot spring waters represent a mixture of heated ground water and magmatic water arising from superheated steam. One source may be enriched at the expense of the other.

Hot springs may be classified in several ways. A simple method is to recognize three principal types: alkaline, acid-sulfur, and calcareous springs. The alkaline type comprises over three-fourths of the earth's hot springs. They are generally rich in sodium, bicarbonate, and chloride. Silica is usually quite high (>200 mg/l) and sulfate varies with locale. The pH may range from about 6.0 to 9.0 and siliceous sinter is the main deposit. Acid-sulfur springs are almost invariably associated with recent or current volcanic activity. They commonly have pH values of less than 3.0 and are initially high in hydrogen sulfide. Sulfur compounds of various oxidation states, including sulfuric acid, abound. They, of course, lack bicarbonate, have little

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chloride, but may also be very rich in silica. Calcareous hot springs are near neutrality and are enriched in calcium, magnesium, and bicarbonate. They have far less silica than the other types. A rich supply of CO_2 results in CaCO_3 (travertine) deposition instead of sinter.

The very acid springs, although often presenting a full thermal gradient, seem to lack all photosynthetic organisms below pH 4 or 5 with the exception of Cyanidium caldarium which inhabits the thermal range from about 60° to 35°C. Cyanidium is certainly not a blue-green alga but has a pigment system resembling that of the Cyanophyta (see Mercer et al., 1962).

Most hot springs, including the abundant alkaline types, are greatly enriched in certain elements which occur in temperate lakes and streams in traces only. Some of these are required micro-nutrients. Many are known to be poisonous to microorganisms at concentrations far lower than those occurring in many hot springs. High temperature, anaerobic conditions, and a source of super-heated magmatic steam contribute to the enrichment of several rarer elements in thermal spring issuances. The continued solubility of most, after the water has reached the surface, is greatly dependent on pH among other factors. Uzumasa (1965) reports the following extreme values of some of these elements in hot springs of Japan (in mg/l): aluminum--1,000, arsenic--5.1, cobalt--2.19, copper--68.0, fluorine--16.0, iron--1,000, lead--2.60, nickel--9.38, manganese--278.0, and zinc--2.00. The greatest enrichment of the metallic elements occurs in the more acid waters. Arsenic concentrations may be high in either alkaline or acid waters. Fluorine is more prevalent in mildly alkaline waters. The median value for fluorine was between 1.0 and 2.0 mg/l in Japan. In Yellowstone Park, values from 15.0 to 20.0 mg/l were common (Allen and Day, 1935). In Japan the mean value for arsenic in 174 neutral or alkaline springs containing the element was 0.28 mg/l. It was about twice this value in 16 acid springs (Uzumasa, 1965). Values of over 2.0 mg/l were common in Yellowstone. In the acid springs of Hokkaido the mean concentration of copper was about 98 $\mu\text{g/l}$ and in the neutral to alkaline springs between 20 and 30 $\mu\text{g/l}$. Manganese levels are higher in the acid to neutral springs but may be significant at least up to a pH of 8.0. The average value for manganese-containing Japanese springs is 2.3 mg/l. Manganese is also one of the enrichments of some neutral to alkaline hot spring groups in Yellowstone. Black MnO_2 precipitates are formed for great distances in the drainways, often well below the thermal range.

There are some reports of manganese toxicity to algal growth at normal limnetic and at higher (hot spring level) concentrations (cited by Wiessner, 1962). The toxic effect of copper on algae is well known even at levels below 10 $\mu\text{g/l}$. The toxicity of arsenic and fluorine

compounds is also well documented. However, the tolerances of most non-thermal and thermal algae to the above elements have not been established. In the few biological investigations of hot springs, analyses of the minor elements present were not made. I am implying, however, that the chemical composition of some hot springs may have a severe excluding effect. Toxic factors may account in part for floristic or productive differences among various hot springs which may appear alike superficially.

There is at this time no evidence to suggest that any of the known micronutrients of blue-green algae occur in suboptimal limiting amounts in thermal waters. Thermal waters, having high salinities in general, might be expected to be eutrophic in a complete sense. Unlike saline lakes, however, which are usually rich in all macronutrients, including combined nitrogen and phosphorus, thermal springs can be quite deficient in ammonium, nitrate, and phosphate. Very minute concentrations, nevertheless, may be adequate in a constantly flowing environment. Unfortunately, few springs have been analyzed for these important nutrients.

In Japan ammonium was detected in less than one-half of the 860 hot springs analyzed, but in those it averaged about 1.6 mg/l (Uzumasa, 1965). Nitrate, also derived directly from percolating ground water or from the oxidation of ammonium, was found in only 15% of the Japanese springs and in those averaged less than 0.3 mg/l. Although detectable in only 37% of the Japanese springs, phosphate averaged about 6.5 mg/l in those, which is a very high biological value. In Hunter's Hot Springs, Oregon, nitrate-N was undetectable at a main source, but increased substantially about 20 meters downstream after passing over algal mats and receiving some drainage water from the shore. Values from 42 to 142 μ g/l were recorded at different seasons at the downstream station where temperature had decreased to 45° C. Phosphate-P also increased greatly downstream (to over 100 μ g/l), possibly from shore drainage, although values as high as 40 μ g/l were found at the source. Brock (personal comm.) found more than 2.0 mg/l phosphate in an alkaline Yellowstone spring in which he is studying productivity. He indicated that there was little change downstream. Values of phosphate as high as those mentioned for Yellowstone and Japan would be considered inhibitory levels for some "oligotrophic" algae. On the other hand, Brock found little bound nitrogen in his spring. N_2 is one of the common gases in alkaline hot springs unassociated with volcanic activity, but may be extremely rare in others. The volcanic (magmatic) gases are generally CO_2 , H_2S , and H_2 . Vera Dugdale (in this symposium) reported active nitrogen fixation in mats of Mastigocladus in an Alaskan hot spring. Sheridan (1966) found no fixation in an axenic culture of Synechococcus in my laboratory (Y50BII). Probably no species of this unicellular genus are capable of utilizing N_2 . Some spring waters

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initially low in bound nitrogen may greatly restrict the development of high temperature Synechococcus strata, while N-fixing algae, characteristic of the middle and lower parts of the thermal range, would not be impaired.

The eutrophic nature of some hot springs is probably reflected by results seen after a number of hot spring drainways have emptied into a small river which drains a normal type of watershed in its upper reaches. The standing crop of algae and macrophytes in the Firehole River, Yellowstone Park, increases noticeably below the tributaries of the Upper Geyser Basin. Greatly increased photosynthetic rates were also measured below these additions (Harrington & Wright, 1966). Presumably any toxic factors would be sufficiently diluted when entering the river.

In general, the thermophilic blue-green algae seem to act as eutrophic organisms. A large variety of species from Oregon, Yellowstone, and Iceland have been cultured in my laboratory in a heavily fertilized defined medium (Table 1). The medium was formulated by R. P. Sheridan in my laboratory, particularly for S. lividus (Sheridan, 1966). It is also used for the experimental work with Oscillatoria terebriformis. The addition of vitamins B₁₂, thiamine, and biotin does not appear to stimulate growth in Synechococcus or O. terebriformis. Sheridan (1966) failed to obtain heterotrophic growth of S. lividus in the dark. However, the lack of heterotrophic ability is the general rule for the Cyanophyta. Among photosynthetic thermophiles it is well documented only in Cyanidium caldarium, in which growth in the light is also stimulated by galactose and malt extract (Ascione et al., 1966).

It would be interesting to have information on the concentration of various dissolved organic compounds at different stations in thermal streams and on the nature and abundance of heterotrophs. The major portion of the gel-like mats of most hot springs, under the thin photosynthetic upper layer, is probably composed of non-chlorophyllous, carotenoid-containing filaments of 1 μ or less in diameter. These should probably be referred to the "flexibacteria" instead of the blue-green algae (see Lewin, 1962). The detailed composition of thermal mats and the reasons for the slow mineralization are of continuing interest.

I would like to acknowledge with thanks the aid of the National Science Foundation grants G-19972 and GB 2951 for work done in my laboratory. I would also like to thank Mr. John Good, Chief Naturalist, Yellowstone National Park, for permission to collect algae in the Park.

Thermophilic Blue-Green Algae

Table 1

Culture medium for thermophilic blue-green algae
(after Sheridan, 1966)

The components are added in the order listed. For normal use the pH is adjusted to 8.2 with NaOH. The turbidity resulting from steam sterilization clears completely in less than 24 hours. The final pH is 7.5. A ten-fold concentrated stock solution is usually used; it is stored at 4°C unsterilized. NaOH is added after dilution and before autoclaving.

Distilled water	-----	1,000 ml
Nitrilotriacetic acid (NTA)	-----	0.1 g
Nitsch's Trace Element Solution	-----	0.5 ml *
FeCl ₃ solution	-----	1.0 ml **
CaSO ₄ ·2H ₂ O	-----	0.060 g
MgSO ₄ ·7H ₂ O	-----	0.100 g
NaCl	-----	0.008 g
KNO ₃	-----	0.103 g
NaNO ₃	-----	0.689 g
Na ₂ HPO ₄	-----	0.111 g

* Nitsch's Trace Element Solution

Distilled water	-----	1,000 ml
H ₂ SO ₄ (conc.)	-----	0.5 ml
MnSO ₄ ·H ₂ O	-----	2.280 g
ZnSO ₄ ·7H ₂ O	-----	0.500 g
H ₃ BO ₃	-----	0.500 g
CuSO ₄ ·5H ₂ O	-----	0.025 g
Na ₂ MoO ₄ ·2H ₂ O	-----	0.025 g
CoCl ₂ ·6H ₂ O	-----	0.045 g

**FeCl₃ Solution

0.2905 g FeCl₃ in 1 liter water (1 ml = 0.1 mg Fe)

NOT REPRODUCIBLE



Figure 1. An aerial mat of thermophilic blue-green algae on the cone of Spray Geyser, the Imperial Group, Yellowstone National Park, in August 1966. The arrow indicates one orifice of the geyser which erupts with considerable spray. The quiet periods last only a few minutes. The algal mat is several centimeters thick in places and consists largely of Phormidium and Calothrix.

NOT REPRODUCIBLE



Figure 2. A high temperature Synechococcus cover in one of the Five Sisters' Springs, White Creek drainage, Yellowstone National Park. The thin yellowish-green cover is shown extending up out of the deep pool. The edge of the cover is at about 73-74°C (arrows). The water in the deep pool is lower than 72°C. Water of over 74°C is flowing over the deep pool from an adjacent pool to the left. August 1966.

NOT REPRODUCIBLE



Figure 3. Sharp species demarcations in Hunter's Hot Springs, Oregon (March). The temperature of the water flowing in the main channels (large arrows) was between 60°C and 54°C. This entire range is occupied by green covers of Synechococcus. The range from 54°C to 47°C is occupied by the dark mat of Oscillatoria terebriformis (small arrows). The length of the area photographed is about 4 feet.

NOT REPRODUCIBLE



Figure 4. A close-up view of Oscillatoria terebriformis (dark mat) occupying a flow pattern on a background cover of Synechococcus. Individual trichomes and tufts of interwoven trichomes may be seen. The temperature of this area (5-6 cm in length) was approximately 53°C. Hunter's Hot Springs, May 1966.

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GROWTH REQUIREMENTS OF BLUE-GREEN ALGAE
AS DEDUCED FROM THEIR NATURAL DISTRIBUTION

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Blue-green algae constitute an assemblage of organisms ranging over a vast array of ecological situations. Their physiological capabilities are more varied than any other algal group. Their ancient origin has permitted much in the way of evolutionary advance, but this has resulted in physiological variants far in excess of morphological types. It would be hopeless, then, to attempt a discussion of environmental requirements of all blue-green algae. This discussion will be limited to those primarily of concern to man in his water supplies.

Relatively few genera of blue-green algae have become important economically, but studies of these have resulted in sufficient information to permit certain generalizations as to environmental requirements. Growth appears to be favored where conditions of low mineral concentration and high organic material content exist. Organic pollution almost always insures an overabundance of blue-green algae. Additionally, excessive blue-green algal growth occurs during periods of high water temperature and light intensity, and these are, of course, related to climate and weather, and also to latitude. These generalities suggest that some areas or bodies of water may become susceptible to troublesome growths whereas others may remain safe indefinitely, or at least until the above conditions are met.

Numbers of organisms are of concern to this discussion. The mere presence of a given species does not cause water quality problems even though a given organism is well known as a troublemaker. It is only in excessive amounts that any organism becomes ecologically or economically important. The environmental conditions that permit mere presence, or existence, of individuals differ from those which stimulate a bloom; but it is possible that the conditions differ only as a degree of concentration of certain environmental factors. It appears to be usual, however, that when the growth curve of a population of blue-green algae starts up, it continues to a maximum.

The identity of an organism, or organisms, becomes important because it is well established that some--but by no means all--species of blue-green algae become involved in blooms or otherwise are of nuisance value. Thus, taxonomy is of importance.

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It has been said that the greatest hindrance to the ecologist is taxonomy. However, this statement may be interpreted as: Even the taxonomist is hindered by the taxonomy of the blue-greens. The problems here are met at the generic as well as at the specific level, and much has yet to be accomplished in providing a system acceptable to most taxonomists. There are now in use basically two approaches to taxonomy of the genera of coccoid blue-green algae (Drouet and Daily, 1956; Geitler, 1932), and each has its followers among the taxonomists. As yet, there is no more recent critical study of the filamentous forms than that of Geitler (loc. cit.). Hopefully, a meeting such as this will provide the encouragement for a concerted effort to update our taxonomy, perhaps by incorporating information from physiological studies and by stimulating cultural studies which contribute to the understanding of range of morphological variability in at least the more important members of the blue-green algae.

Any consideration of distribution of organisms should include the geographical aspects, or patterns of distribution. Apart from the purely descriptive results, clues to environmental requirements might then be forthcoming. It is useful to describe species distribution by the terms endemic, disjunct, or cosmopolitan, depending in turn on whether they are limited to a very small geographic area, are found in a few scattered localities but are absent between, or whether they are universally found. Each of these suggests genetic adaptation to an environment of a very restricted sort, or to most environments. But here we lack information on distribution--except for troublesome occurrences which get the publicity--and almost nothing is known about blue-green algal genetics, even of the troublesome species. It is probably true at this time that any summary of our knowledge of geographical distribution would only expose the distribution of the blue-green algal taxonomists.

Another aspect of distribution is that of succession, or distribution in time. Some species are found in the same body of water whenever sampled, but others may be seasonal or of sporadic occurrence. This, of course, complicates the mapping of patterns of distribution. The possibility of migration must also be considered, and the environmental changes as wrought by man will continue to have their influence.

Some specific cases may be useful in illustrating some of the problems just outlined, and, hopefully, they may suggest new means of approach.

First is a problem of taxonomy at the generic level. During the summer of 1964 a group of us from the University of Montana Biological Station observed a bloom of Gloeotrichia echinulata in a small lake (Rainbow Lake, formerly Dog Lake, 50 miles southwest of Flathead Lake).

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Except for its planktonic habit, this species here differs from other Gloeotrichia in lacking akinetes (gonidia), and this characterizes Rivularia when present. Some authors, incidentally, include the two genera under the name Rivularia. Blooms of Gloeotrichia in this lake have been observed in successive summers as well.

I have observed a case of a more or less continuous bloom of blue-green algae (with Botryococcus) for a period of five years in Freshwater Lagoon just south of Orick, California, on the coast. This lagoon is separated from the ocean by a sand bar, along which U. S. Highway 101 passes. Apparently, marine water does not get carried into this lagoon. The blue-green algal components of the bloom include the genera Microcystis, Coelosphaerium, and Anabaena. Limnological data are not available, but it is notable that this condition occurs in a relatively cool coastal environment.

A situation with many ramifications is illustrated by the algae in oil field sump ponds, on which, apparently, nothing has been published. I have discovered a rather startling array of algal types associated with crude oil and tars in investigations over a period of years in the States of Oklahoma, Kansas, and California. A few observations seem pertinent here, but the details will be published elsewhere.

Blue-green algae predominate in these habitats, often to the exclusion of other algae (diatoms, green algae, Trachelomonas spp.). In one Kansas sample which appeared to be a semi-solid tar from the bottom of a sump pond, Schizothrix Friesii Gom. made up the bulk of the "tar," with but few water droplets apparent. This species, incidentally, is a common soil alga in Kansas. At the opposite extreme of substrate "texture," more or less clear samples contained minute oil droplets in suspension with diatoms entrapped within, but these samples lacked blue-green algae.

Floating "tarry scums" from California^{1/} contained predominantly Oscillatoria spp. and clearer samples included coccoid blue-greens.

The algal growths in oil field sump ponds may be conspicuous, or they may not, as compared to the substrate. When apparent as floating scums, the color ranges from pale blue-green (pea soup color) to yellowish or brownish. When not apparent, as in the benthic tar Schizothrix mentioned above, the algae may be present in abundance.

^{1/}The California collections were made with the help of, and studied under contract with, the California Department of Water Resources, San Joaquin Valley Drainage Investigation.

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There were significant differences in algal floras depending on whether the oil field contained brine water or non-brine ("freshwater" might not here be equivalent). The blue-greens predominated in the non-brine oil fields.

Though quantitative measurements were not made (methods have yet to be devised), certain deductions appear to be worthy of consideration. It can be presumed that a minimal amount of light, or none, can penetrate the medium, though this will vary with the relative concentrations of water and oil or tar. It appears that certain blue-green algae can thus thrive in crude oils in the absence of light. Furthermore, there appears to be no possibility of diffusion of gases into, or out of, the medium.

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RECENT ADVANCES IN THE
PHYSIOLOGY OF BLUE-GREEN ALGAE

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I would like to reschedule the topic of this talk from "Recent Advances in the Physiology of Blue-Green Algae" to just "Odds and Ends," because that is just about what this is going to be. Having been cloistered for eight years in a temporary building at the University of Wisconsin with George P. Fitzgerald, I had no idea that so many blue-greenologists could be assembled at one time in one place. It is indeed a most pleasant surprise to find this. Another surprise not so pleasant to me, however, is finding myself on this platform right now. I had withdrawn my contribution for this occasion several months ago, but due to a bad confusion in the U. S. mail and to my being away in Tokyo, I had not planned to be here. But then at the last minute, I decided to come and listen to the speakers and absorb a lot of good information and meet old friends. I arrived last night and was rapidly persuaded by Drs. Dollar and Oglesby to present a few remarks which might be slightly provocative to a few people. I hope they are. I also hope that you will excuse any serious omissions I might make due to this completely off-the-cuff presentation and correct an erroneous statement or concept which I might give forth.

In addition to the reasons for studying blue-green algae which were stated this morning, I would like to add a few of my own. First, due to their cellular organization, their chemical composition, and many of their physiological attributes, the blue-greens, as you all know, occupy a position of great importance and interest in regard to questions of phylogeny and evolution of higher plant forms. In this connection, I would like to call your attention to the numerous statements which you hear and read in the literature and textbooks and reviews concerning the blue-greens--the relationships between blue-greens and the colorless blue-green algae, and to such organisms as *Cyanidium* and *Glaucocystis*, and to the rather peculiar and ubiquitous group of organisms which Ralph Lewin and Soriano have written quite a bit about, the flexibacteria. The relationships within this great assemblage of microorganisms demand some very good analytical chemical work in the future to either reinforce or to refute the proposed closeness of all this vast assemblage of microorganisms, both photosynthetic and colorless forms. In view of the obligate autotrophic nature of many blue-green algae, a subject which I will discuss later on today, I personally find many of these

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assumptions and statements in this connection about the closeness of all these microorganisms to be rather questionable. It remains for further analytical work to show which way the phylogenetic pathways will go.

The second major area for studying blue-green algae, in my estimation, is to obtain information on basic problems in molecular biology. Now, for problems in molecular biology, you might as well use E. coli or Chlorella or a spinach leaf obtained from the local A&P supermarket as long as that does the job. However, if a blue-green algal cell can supply some special characteristic or information to your problem, then the blue-green is advantageous to use, of course. In this connection, I would like to run through about ten various problems in microbiology where I think blue-greens have contributed, and will continue to contribute, much information. The first one is involved with the basic morphology and function of the blue-green cell. As you all know, the basic structure of the blue-green is very similar to that of bacteria. It is a procaryotic cell and does not have the organized organelles of all higher plants and animals. In all eucaryotic cells, you have the packets of enzymes organized in such organelles as the mitochondria, the chloroplasts, the nuclei, etc. Evidence is now accumulating that the enzymes are oriented very specifically so that substrates can be passed down along the whole chain of enzymes much in the fashion of a zipper. Now what happens in the case of blue-green algae? We don't have any organelles there. If you look at the microscopic picture of blue-greens, what do you see? You see the various layers surrounding the cell, you see an ill-defined peripheral portion containing many lamellae, and a nebulous area of the centropasm, which is very often traversed by the lamellae which are found predominantly around the peripheral portion. But they do transverse the central portion as well. Where do you get the packets of enzymes and how are they oriented in the blue-greens compared with all higher plant and animal cells? Now, this is a question which is of the utmost importance in cell growth and differentiation. With the blue-greens, unfortunately, it is very hard to study this problem because, for one thing, you break the cell and you cannot isolate nice, clean entities such as chloroplasts, mitochondria, or a nucleus. So the nature of the problem is exceedingly difficult, and although many people are interested in it, I know of no good data which have been accumulated, or do I see any real hope for achieving any breakthroughs in this area.

The second area involves gliding movements of many of the blue-greens, most fascinating movements to watch under the microscope, as many of you know, but also it is a phenomenon which is not explained at all. There is little evidence as to means of locomotion as there are no flagella or cilia, and no muscular threads of any kind. It is

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apparently associated, as we heard this morning, with phosphorylation and ATP, but the basic mechanism of how a filament can glide through agar or wave back and forth is entirely unknown. It is an extremely interesting problem.

Another area is nitrogen fixation. Here the blue-greens have contributed quite a bit of information. Blue-green cells have been broken, and cell-free systems have been isolated by the Wisconsin group of Burris, Wilson, Singh, and Nicholas in which they have actually gotten nitrogen fixation. One interesting point which has come out is that cobalt apparently is intricately involved in one of the early steps of nitrogen fixation, although it is not pinpointed at all, as yet. It is extremely difficult to get at because of the lack of any suitable radioactive isotope of nitrogen. They use ^{15}N , which is the heavy, stable isotope.

Another area, the fourth one, is the function of accessory pigments in photosynthesis, an area in which the blue-greens and some of the reds have contributed immensely to our understanding of the physical chemistry and biochemistry associated with photosynthesis.

A fifth area is the question of the seemingly obligate autotrophic nature of all blue-green algae. Many of you will disagree with this statement, but please wait until the end of the talk to question me on this point. But for now, I'll call them obligate photoautotrophs.

Another area involves the microelements and nutrition of blue-green algae, problems which have taken a lot of time for many investigators. It looks rather routine to me for the essential macro- and microelements, with one exception. That is the situation with cobalt, where cobalt has been demonstrated to be an essential microelement for all blue-green algae which have been investigated. It is implicated in nitrogen fixation by Burris' and Wilson's results, and also by my earlier results at Wisconsin. It might well play--in fact, I'm quite sure it does--some non-nitrogen-fixing role in the blue-greens. It is required by Coccochloris, by Microcystis, and many of these cells that don't fix nitrogen, so it is involved in a number of metabolic sequences, such as in methylation, nucleotide reduction, vitamin B¹², etc.

Another area, the seventh one, is the area of the excretory mechanism and the excretory product of blue-green algae. Here we get into a factor which is very important ecologically in that the blue-greens, apparently from laboratory studies, at least, do excrete lots of organic metabolites from the cell back into the extra-cellular solution. How they do this is not known, and in addition to the oxidizable

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substrates they kick out, there is also the very important factor of the toxins they produce which, as you know, can be economically very important.

The eighth area is the effects of light in a non-photosynthetic mechanism. There is evidence from Lazaroff that light also plays a role in the differentiation of the cell and in the completion of what he calls the sexual life cycle of blue-green algae. To my knowledge, his work has not been duplicated or repeated successfully by anybody. It looks like nice work to me, but a lot more work has to be done to know exactly what is happening here.

In line with that, there is the question of how the genetic information (the DNA) is passed along from generation to generation with such consistency in the blue-green algae. There is no mitosis, as we know it in higher plants and animals, and the exact mechanism of this division of DNA between two daughter cells is not well understood. The question of sexuality is also one which is not at all understood; most textbooks claim that they are asexual, putting the poor blue-greens in a rather dismal pile on the bottom. People like Lazaroff claim that they do fuse and engage in sexual transformations. Again, this has not been duplicated, to my knowledge, by anybody else. That was mostly a microscopic examination, but I'm still waiting for more work before I decide for myself whether that is really so or not.

Another area is the area of cellular differentiation where I think blue-greens are very promising organisms for experimental work. As you know, there are several types of vegetative cells, akinetes, heterocysts, various spores, and the formation of these can be controlled to some extent. There has not been much experimental work along this line, but I think it is a very fruitful one for future investigators.

Well, these are some of the areas I wanted to talk on, but then last night after saying I'd chat for a few minutes I read this brochure, and I read that we are supposed to limit our comments to the environmental requirements of blue-green algae, so I realized that I would have to dispense with all those former topics and talk about something else. So I decided to say a few words in the remaining minutes on six subjects--it's going to be fast, though. One is temperature, the second is desiccation, the third is light, the fourth is nutrients, the fifth is pH, and the sixth is heterotrophy, on which I'll spend about five minutes.

In regard to temperature, we heard quite a bit from Dr. Castenholtz this morning on the effects of high temperature, so I will say nothing more about high temperatures. As the temperature goes down and passes through the zero centigrade point, there are two major areas of concern

to the cell. One is with decreased temperature; there is a decrease in the enzymatic rate of all the chemical reactions and the various metabolic patterns and routes can be upset, which can lead to damage or death of cells. The second major area of concern to the cell would be the fact that when the freezing point is reached there is a phase change where liquid water freezes to give the solid state. This is the crucial point, the most damaging point of freezing damage, it appears. First, there is the possibility of deformation or physical damage from rupture of the cell due to ice formation. Secondly, freezing out of solutes occurs so that there are osmotic changes within the cell which can lead to denaturation of the proteins, etc. So for the initial phase change, it is a period of dramatic concern and potential damage to all cells. As you heard this morning from Dr. Castenholz, I collected quite a few blue-greens, greens, and diatoms in the Antarctic about four or five years ago; during the past few years, I have used these isolates plus about 20 species isolated from the Wisconsin lakes, in low-temperature studies. I studied them in regard to survival after rapid freezing versus slow freezing, retention of viability after freezing, thawing, freezing, thawing, up to 15 or 20 times. All the Antarctic forms I isolated were, as you would predict, very capable of surviving the freezing and thawing process. Of the ones isolated from the Wisconsin and Midwest area, a good many showed fairly good survival. Some of them, such as *Nostoc*, were very good; they were as hardy as anything I got in the Antarctic. Many of the ones, such as *Phormidium* and *Oscillatoria*, were horribly sensitive; they would not survive even one freezing. Many from the Antarctic isolates, such as *Schizothrix* and *Nostoc* and some of the *Anabaenas*, could be frozen and refrozen 19 or 20 times, with no difference in the apparent viability. Many of them could be frozen with liquid nitrogen, or slow frozen by gradual decline from zero to minus 15 or 20 degrees. Most of the Antarctic ones survived slow freezing better than rapid freezing. This is true for nearly all the temperate as well as all the polar algae I worked with. With slow freezing there is less damage than with rapid freezing, which is rather contradictory to what you would expect and predict by reading the elementary textbooks. Slow freezing leads to bigger ice crystals and potentially more damage, you would think, but it apparently does not work that way. One other interesting thing about the blue-greens is that the ability to survive freezing is not aided in any way by the addition of organic adjuvants to the suspension of cells before freezing. This is in sharp contrast to all the greens, where the addition of horse serum or glycerol, glutamate, milk powder, etc. will increase very greatly the number of cells that survive. But with the blue-greens, it has absolutely no effect. You can do it in distilled water or horse serum or blood, or anything else that you like, and the results are always the same. In regard to freezing damage, Levitt has theorized on why some cells are so capable relative to other forms of life in regard to surviving freezing. It involves the sulfide-disulfide linkages and

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the fact that you apparently have fewer new intermolecular disulfide bonds formed by the proteins of low temperature resistant cells. There is some experimental evidence for this, and it is a rather interesting and fruitful area for further investigation. In this regard, I was going to ask Dr. Castenholz this morning about speculations on the characterization of the proteins which enable them to survive 90°, but I got too hungry, so I'll have to see him tomorrow. So much for topic one.

Dessication from the frozen state is of immediate concern in both Polar regions. Freeze-drying involves first freezing the cells and then sublimation of all the water molecules directly from the solid state to the gaseous state. In this way, drying can be effected with minimal shrinkage and deformation of the tissue. Unless the initial freezing has resulted in damage to the cells, the cells should be undamaged. Theoretically, it should be possible to freeze-dry almost any cell if you can get rapid enough temperature gradations to inner parts of the cell. I have worked with both Antarctic and Wisconsin algae a great deal in connection with freeze-drying. In the Antarctic this is a natural phenomenon and a danger which cells have to face every day of the year the whole year round. The microclimate is extremely important, depending on whether the sun is shining, whether the cell is in the lee of a rock, etc. The low air temperatures coupled with the very low humidity in the atmosphere result in a freeze-drying of any cells exposed to the atmosphere. My laboratory studies with the Antarctic blue-green algae indicated that most of them were capable of surviving freeze-drying very well. All these tests, by the way, are of necessity semi-quantitative because I have not been able to plate out any unicellular blue-green alga and get a 100% recovery of the number of cells. In other words, if you plate out 100 cells of *Anacystis*, you cannot get 100 colonies as you can if you plate out 100 cells of *Chlorella*, plus or minus 2 or 3% for the statistical error. But with blue-greens I have not found any method for doing this, so I place the sample into a liquid culture in an Erlenmeyer flask and examine it once or twice a day and count the days until the growth becomes visible. This is roughly proportional to the number of viable cells, and this is a very rough, semi-quantitative procedure, but it is the best we have available until somebody can tell us more about how to culture these tenacious blue-greens. For any filamentous blue-green algae there is the additional problem of how to separate the whole chain of cells into a unicellular suspension. One interesting thing about a freeze-dried cell is that the ability to survive high temperature is greatly increased. I can take lyophilized samples of my blue-green algae and heat them at 100°C for over an hour and rehydrate them, and they will have undergone no damage during that heating process. In the hydrated state they cannot survive 35 or 40° C for a few minutes.

I would like to add just a few comments on pH because we had quite a bit of discussion this morning and I hope we have more tomorrow. Blue-green algae require very different pH conditions than do most other types of algae. Some blue-greens will grow at pH values down to about 7.0, but the optimum pH range seems to be about 8.0 - 10.0. In some healthy laboratory cultures the pH has been measured very close to 11.0. Such a high pH requirement is interesting in regard to the question of the form of inorganic carbon utilized in photosynthesis. In other algal groups it has been shown that CO_2 or bicarbonate is preferentially assimilated during photosynthesis. By reference to a curve showing distribution of CO_2 , HCO_3^- , and CO_3^{2-} as a function of pH (e.g., see Photosynthesis by Rabinowitch, Vol. I, p. 177), it is clear that at pH 11 there is extremely little free CO_2 , a few percent bicarbonate, and a high concentration of carbonate ions. Does this mean that the blue-green algae are assimilating carbonate ions preferentially, in contrast to most of the other algal groups? To my knowledge there has been no work on this problem with the Cyanophyta, so I cannot give you any answer to this question.

Concerning light, one thing that has always bothered me in the laboratory with blue-green algae is that you have to use low light intensity. With all the blue-greens I have grown I have used a light intensity less than 1000 ft.-c., and usually about 100 or 200 ft.-c. If you have a very dense suspension of Nostoc, which will attenuate the light markedly, a much higher light intensity can be used. But with a dilute suspension relatively low light intensities must be used. Now this disturbs me very much because in their natural environment they are exposed to very high light intensity of 8,000-10,000 ft.-c. This contradiction between laboratory growth and field occurrence is a troublesome one, and to me it indicates that our culture methods are probably pretty lousy--that we are not obtaining healthy, normal cells, but are obtaining pretty debilitated, semi-sick cells. The great change in morphological shape of many blue-green algae from a lake to a laboratory would also lend evidence to this conclusion. I hope I don't step on anyone's toes when I describe your cultures as sick cells. But there is--I think most of you will agree--the fact that morphological appearance of cultured cells, cells which have been cultured for a long time in the lab, really do deviate quite a bit from a nice, healthy cell which you pick out of the lake. So much for the light.

The next section is nutrients. Again, to my knowledge, there is no organic nutrient requirement for any blue-green alga. In regard to inorganic nutrients the essential major elements (C, H, O, N, Ca, Mg, S, K, P) are the same for all organisms. Some people might question the calcium requirement, but I would be prepared to put up a good argument for that one. The essential microelements are iron, boron, manganese, molybdenum, copper, zinc, and cobalt; to this list I think chloride

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and sodium should also be added. There are a number of other elements which may be essential, but no convincing case has as yet been made for any of them with blue-green algae. Manganese is a rather interesting nutrient in regard to pigmentation. In a Mn deficient culture of Nostoc muscorum the chlorophyll content is extremely low while the phycocyanin content is more or less like the normal cells; the cells thus have a distinct bluish color. Boron, molybdenum, and cobalt also cause changes in pigment complement, but the changes are not as striking as with Mn deficiency.

Concerning the quantitative requirement of all the inorganic nutrients, George Fitzgerald is following me on the platform; from my knowledge of George, I am sure he will do a thorough job there, and I'll dismiss the subject with that.

We then get to heterotrophy, which is the last thing I'll be talking about. Many years ago, back in the twenties and thirties, people like Harder, Allison, Hoover, and a handful of other people, published some rather nice work claiming that various blue-greens, particularly Nostoc and Anabaena, could grow in the dark. This information, coupled with the occurrence of blue-greens in Nature, where they are very commonly found in water which is very rich in organic nutrients, such as we have heard several times today, led to the general belief by laymen and many phycologists and biologists in general that nearly all, if not all, the blue-green algae are capable of both autotrophic or heterotrophic growth. Now this statement has crept into all sorts of reviews and long essays on blue-green algae. I fooled around with it for many years, because I got slightly perturbed when investigators would write that they grew Nostoc in the dark without giving any data and no good description of exactly how it was done. This was done by many well-known people in this country and in other countries without any published data; I would like to see if anyone can give me any data, either today or tomorrow, in favor of heterotrophic growth in complete darkness of any blue-green alga. For quite a few years now I have used quite a few different species of blue-greens and a great variety of organic substrates, and the net result is that I have found absolutely no heterotrophic growth as long as you have complete darkness.

Now, in the last few minutes I would like to give you some information which I think might well explain the many discrepancies in the literature regarding heterotrophic growth. To a number of flasks containing inorganic nutrient solution add 1% (by weight) glucose or sucrose to half of them; inoculate them all then with a small inoculum of a blue-green alga such as Nostoc muscorum and place them in complete dark or in light (200 ft.-c.) for one month.

There is no measurable cellular material in any of the dark flasks; the inorganic flasks in the light will have about 200 mg dry weight (per 400 ml suspension), while the glucose-fortified flasks in the light will have about 400 mg dry weight. This is a very dramatic increase caused by the addition of glucose (or sucrose), but I noticed in harvesting these cells that they were very gelatinous. The usual procedure in obtaining such dry weight was to centrifuge, decant the supernatant, and then wash the cells once with distilled water before centrifuging again. As the light-grown cells with glucose were so gelatinous, I wondered if the increase in dry weight might be accounted for by the presence of large amounts of extracellular material which could be washed off by thorough rinsing. In another experiment similar to the above one, some of the light-grown cultures with glucose were washed eight times with distilled water. This caused the dry weight to drop from 400 mg to about 250 mg, which, however, was still significantly higher than the 200 mg in the inorganic flasks in the light. There was no fixed nitrogen source in any of the above experiments, so that all cellular nitrogen was derived via fixation of molecular nitrogen. In addition to dry weight of the cellular material noted above, I also ran Kjeldahl nitrogen determinations on all samples. Results were as follows: all samples in the dark, 0; inorganic cultures in the light, 10 mg N; cultures with glucose in the light and rinsed once, 12 mg N; cultures with glucose in the light and rinsed eight times, 12 mg N. I concluded from these experiments that glucose was not being assimilated in complete darkness, and that in the light it was penetrating the cell and affecting the metabolism of the cell such that more molecular nitrogen was being fixed, which led to a greater dry weight yield per unit volume of suspension. I did a little work with long dark periods (23 hours) and short light periods (1 hour) which indicated that continuous light was not necessary for assimilation of the glucose; periods as short as 30 minutes per day gave substantial amounts of growth.

I also examined the rate of assimilation of organic substrates by blue-green algae by the use of radiocarbon-labelled compounds. After incubating *Nostoc muscorum* with C^{14} -labelled glucose or sucrose for one month in the dark, there was no measurable amount of radiocarbon incorporated into the cellular material. When light was given to these cultures (either continuously or for a short period of each day), the cellular material incorporated much of the radiocarbon from the organic substrate. When the cells were hydrolyzed and the products chromatographed, it was seen that the radiocarbon was distributed in a wide variety of amino acids, carbohydrates, and organic acids.

All this leads me to believe that all blue-greens that I have looked at are obligate autotrophs, but that they can assimilate organic compounds at a very slow rate by a photoactivation process, which we do

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not understand at the present time. There is some direct evidence of this by the students of Watanabe at the Institute of Applied Microbiology in Tokyo. In view of this rather harsh conclusion, harsh to some of you, at least, it is advisable that studies be done with blue-green algae which have been isolated from lichens and also with a variety of blue-greens isolated from the roots of cycads and other rather peculiar places where you would almost certainly expect heterotrophic forms of life to exist. At the present time, however, no such data are available as far as I know. Thank You.

DISCUSSION

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It seems kind of silly to get up here and tell all these people about blue-green algae after Dr. Holm-Hansen talked. He knows everything that goes on at Wisconsin. Luckily, we've done a few things since he left that he didn't touch on. I suppose we have to do a few things besides freeze-dry mice around there. In order to prepare for a discussion session, I listed some of the problems to be resolved, and I think everyone of these problems has already been brought up. But, I'll bring them up again.

One of the problems is the availability of nutrients, how do you know if nutrients are available, where are they coming from, and what is their importance? One of the first ways of measuring this is by nutritional bioassay, and one of the methods is that which has been mentioned by Skulberg in Norway. The bioassay technique, which came down through a long series of workers, consists of adding different nutrients to a sample of lake water or river and finding out which ones stimulate the growth of algae, and saying, "Ah, ha, phosphorus in this area of the river was limiting to the growth of algae because when you added phosphorus the algae grew! Down here below a source of pollution where phosphorus is present, there was no stimulation due to the addition of the phosphorus." Whether you believe in this technique or do not believe in it, at least you can compare different waters and find out what is the effect of adding nutrients to this water. This nutritional bioassay type of thing is something that I think is just in the very exciting phase and could be expanded considerably. We can get some very valuable information from this type of an assay, but one of the problems involved here which some people forget is the fact that you are studying the nutrition of only the algae in the Erlenmeyer flasks that you're working with. Unless you work on the microscale of increase in growth, you would lose the effect of the addition of nutrients. A heavy bloom in Wisconsin lakes means you can't see your hand in six or eight inches of water. This amounts to 5-10 mg/l, while 5-10 mg/l in the laboratory--I mean, you'd think you'd sneezed in the bottle, that's all the growth there is. You can just barely detect this amount of growth. So, if you add one milligram of phosphorus or a tenth of a milligram of phosphorus and wait to find out how much growth you get in dry weight or optical density measurements, you are expanding your data. What you're

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actually finding out is the amount of growth you'll get with the addition of one milligram of phosphorus, whereas in the lake or river that you're dealing with, you would get that same amount of growth increase with 1/50 or 1/100 that amount of phosphorus. In other words, you've got a concentration factor in the laboratory of 50 to 100 times, and, actually, results of these bioassays may not be related whatsoever to what may take place out in the field, except in a very rough, qualitative situation. In the field you're dealing with perhaps 2 or 3 mg/l concentrations of algae and adding minute quantities of nutrients. In the lab you're adding wholesale quantities, you might say, of nutrients, and you're dealing with many milligrams of algae per liter.

There has been some very nice work in England by MacKereth and co-workers where they've actually done all their work with cell counts. They were concerned with finding out how much growth would take place when you take algae from the bottom water versus the surface water. It wasn't a matter of milligrams growth, I mean, they were measuring the counts--the actual algal count--increases. This type of a bioassay where you're actually measuring algal counts of from maybe 10,000-15,000 per liter is a more realistic approach than the type of work we do with our milligrams per liter type of measurement. So there are some problems with nutritional bioassays, but it is a technique that I think will be very good in the future.

One of the things that has been going on recently is to study the nutrition of algae by their chemical composition or by enzymatic analyses. Bar-Akiva, in Israel, has been using enzymatic analyses to determine whether certain types of citrus trees are limited by the available nitrogen, manganese, molybdenum, and iron. We at Wisconsin have been doing this same thing in phosphorus nutrition. We found that the alkaline phosphatase activity of algae is extremely high in algae where phosphorus is limiting the amount of growth of algae. The alkaline phosphatase of algae with surplus phosphorus is relatively low: a difference of 20- to 40-fold activity. My interpretation of why enzyme induction takes place is the fact that when algae are phosphorus-starved, the algae get hysterical, like students before an exam, and seek other methods of getting phosphorus. They increase the alkaline phosphatases in order to break down other sources of phosphorus: the polyphosphates, the metaphosphates, and the organic phosphates. Alkaline phosphatase analysis is a matter of just taking a pill from a pharmaceutical company and dropping it into a suspension of algae. The supernatant will turn yellow or pink after incubation if there's any alkaline phosphatase activity. Theoretically, you can go to a lake and can find out if the algae in that environment are limited in their growth by the amount of phosphorus present. If there is no alkaline phosphatase activity, supposedly they had a surplus

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amount of phosphorus in their environment for the amount of algae that was produced.

Before we found out about this cute little alkaline phosphatase analysis, we were extracting algae and fractionating the phosphorus of starved versus algae with surplus phosphorus. We tried to find out how excess phosphorus had been stored in algae and to see if we could detect it. We don't know what the storage phosphorus is, but we know if we take algae that have a surplus amount of phosphorus available to them and kill them--we boiled them--we will find orthophosphates in the supernate or the extract. In algae that have a limited amount of phosphorus, little or no orthophosphates are extracted. We do not say that there is orthophosphate in these algae (in the literature they talk about polyphosphates, metaphosphates, and metachromatic granules), but we know if we just kill the algae (and it doesn't make any difference whether you boil them, kill them with Algimycin /an algicide/, or freeze them) as soon as the algae are dead, orthophosphate comes out of the algae if phosphorus has been available to the algae in surplus quantities. So we have a system by which we can extract algae and find if there is a surplus amount of phosphorus present. At the same time, we can take another sample of algae and add either a commercial alkaline phosphatase pill or paranitrophenylphosphate and if alkaline phosphatase is present, phosphate will be split off and you'll have paranitrophenol, which under alkaline conditions, gives a bright yellow color. You put some algae and a pill in a bottle, wait 30 minutes at 37 degrees, and it turns yellow. It's very simple test. This works on the diatoms, blue-green algae, and green alga that we tested in the lab. All the algae in the Madison area last summer had surplus amounts of phosphorus. This is reasonable, as the most fertile water in the country is probably in this good dairy land of ours in Wisconsin. What we have often wondered was, in Nature, is there such a thing as phosphorus-limited algae cultures? Luckily, this last summer when we were checking several lakes in Wisconsin to see if there is an algal community that is actually limited in the amount of phosphorus available to it, every once in awhile algal samples would give tremendous activities of alkaline phosphatase in the same sample as high, extractable phosphorus. We found a nice example of this in a sample from Lake Monona, one of our Madison Lakes, where there was a mixture of what I would call Anabaena and Microcystis. So, I went back and sat on a pier with a long glass tube and sucked up an Anabaena colony and put it in one bottle, and a Microcystis colony into another bottle, until I had a nice, green soup in each bottle to take back to the lab. We actually had fractionated the bloom: about 98% Microcystis and about 98% Anabaena. The Microcystis had extremely high extractable phosphorus, but no alkaline phosphatase, and the, supposedly, nitrogen-fixing organism (Anabaena) had low extractable phosphorus and very high alkaline phosphatase activity. Thus, peculiar results obtained with

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mixed blooms could be separated out because there were two different types of species there. Now, the question I raise is a most natural question to ask this group: Where do algae absorb their nutrients? I don't believe that algae absorb their nutrients in the surface waters where they're growing or we say they're growing. I think they absorb them down in the bottom waters. I don't know why they go up, but I think when the algae get to the surface, they no longer are concerned with nutrition, and they're up there for some other reason. Now, this work with nitrogen-fixers and non-nitrogen-fixers, I think is logically open to question. If *Microcystis* and some of the other algae in our area absorb their nutrients in bottom water and they come up to the surface, they'll run out of some nutrients, obviously. I always assume it's going to be nitrogen before it's phosphorus. If they run out of nitrogen, all the surplus, stored phosphorus they pick up down in the bottom waters will still be surplus phosphorus in the surface water if they do not continue to grow. If the nitrogen-fixer comes up from the bottom, it absorbs the same amount of surplus phosphorus (and we know a nitrogen-fixer can have a surplus amount of phosphorus because we've tested it in the lab). If they were to absorb the same amount of phosphorus in the bottom, then come up to the top, and use up all the nitrogen they have absorbed, they would be forced into a nitrogen-fixation situation. They could then utilize their surplus stored phosphorus up to the point where they were actually limited and they would start seeking other forms of phosphorus. So this is one of the theories I've had: that algae do not rely on the nutrients in surface waters of the lake--there probably aren't any as far as I'm concerned. They have absorbed the nutrients from bottom waters that they're using for growth. But it was very fortunate that only the nitrogen-fixers in our very eutrophic lake in Madison were the ones that were actually limited, by my measurements, by their phosphorus content. Well, this is the question: Where do algae absorb their nutrients? And until we really know, we really don't know too much about what are the environmental factors taking place in the lake where we usually find the algae. Whether the nutritional absorption is important in the epilimnion or the hypolimnion, this is one of the major questions for answering in the algae field. It's touched on every once in awhile by people like Lund and MacKereth, where they've taken algae from the bottom and found that such algae would produce more cells than the algae from surface waters. Maybe this is where they get their nutrients, and when you take them from the environment, they might be more viable or have more nutrients. We know if we want to culture algae in the laboratory, it's much easier to take a bottom water samples, bring it into the laboratory, and wait for the little tiny green spots to show up than it is to try and get algae from the epilimnion to grow. I guess we've readily cultured every bloom-forming organism that appears in the Madison Lakes by using bottom water, whereas only one out of a hundred cultures would grow from

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the surface or upper part of the lake. The algae in the bottom are entirely different from the surface algae, but I don't know about the quantity involved. Are there really enough algae at the bottom with nutrients in them to create these dense blooms in the surface? I don't know. But this is one of the questions that we could talk about further.

One of the questions brought up recently, and touched on today, was the question of nitrogen fixation. I've often wondered if the nitrogen that is fixed is more available for rapid degradation or availability to algae than the nitrogen from green algae or diatoms. If you put algae in the bottom of a tube, the release of nutrients can be measured. The phosphorus comes off very rapidly if the algae are killed and if you do various things to these algae to try to leach them they die. But the nitrogen usually doesn't come out, or very little nitrogen would come out, of these algae. If this is the case with the nitrogen-fixing organism, then its nitrogen really isn't very available to other algae without a long period of time for degradation. The question I would like answered eventually is: If you took a nitrogen-fixing organism and Chlorella or a desmid and did this same type of an experiment, would you find that the nitrogen would be released? We know phosphorus would come out very rapidly, but would the nitrogen come out at a different rate from the nitrogen-fixers than from the green alga? This is something I hope you will be able to tell me.

I was so happy to see Dr. Prowse here because I thought that he could solve all the problems about temperature. Here's a man that doesn't have to worry about temperature. It doesn't vary, and yet they have nice, beautiful blooms of algae. (I am a little prejudiced. To me the nice, pea-soupy, bluish-green color of a lake is beautiful.) They have continuous blooms in the tropics and so can eliminate many of the things that we worry about: temperature and weather changes. The algae grow there despite these things and it kind of knocks in the head many of the theories on effects of weather. One of the questions I would like Dr. Prowse to talk about, and maybe Dr. Hammer from Canada, is: From farm ponds and eutrophic lakes we get vague references to the fact that people, who are using these farm fish ponds for the culture of fish and actual production of a utilizable harvest of fish, can control the growth of algae to the species that they want. I've never had any details and I never knew what species they want in the way of blue-green algae in these fish ponds, but I've heard vague references to the fact that by the proper fertilization of these ponds they can eliminate the Aphanizomenon or increase the Aphanizomenon, whichever it is, and get a specific type of algae that they want. This is the question for Dr. Prowse. I hope this is going to be a thing of the future. If a fish farmer can tell us how to control the growth of Aphanizomenon and lead it into Anabaenopsis, this might be a very good ecological clue for

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us to go on. Quite a few people have mentioned the fact that these beautiful blue-green algae blooms that we get in reservoirs and lakes are usually associated with sewage effluent or most frequently associated with sewage effluent. Perhaps we could control the obnoxious forms (other people call them obnoxious forms), the floating blue-greens, as opposed to the algae causing green water. Pea soup green water is not objectionable, except to water treatment people, but the floating blue-green algae do cause some objections. If you could control them or know what it is that causes their ecological control, we might be able to shift the balance from one species to another.

Well, I think this has brought up questions that we can put back to the speakers that have already spoken or some of the people who haven't. Well, Dr. Prowse, why don't you start and tell us something about fish ponds and lack of temperature changes.

SUMMARY OF FLOOR DISCUSSIONS

summarized by

Jerry C. Tash
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Discussion following paper
"Why Study Blue-Green Algae?"
by W. T. Edmondson

At the Tropical Fish Culture Research Institute, Malacca, Malaysia, Tilapia and Silver Cod are well-known fish that feed on phytoplankton. These fish can eat and digest Anabaenopsis but most of the cells of Anabaena pass through the digestive tract intact. In ponds where fish feed predominantly on Anabaenopsis, algal blooms never develop, but when Anabaena takes over (usually when high phosphate conditions occur), pea soup blooms occur. Little is known about the mechanisms involved in the resistance of digestion by algae but the ability obviously has evolutionary advantages.

Increases in blue-green algae often occur in lakes after sewage is introduced, but blue-greens seldom are a problem in oxidation ponds. The causes for this difference are unknown.

It is important to be aware of the immediate past history of algae when trying to evaluate the standing crop, because the standing crop is partially a result of the rate of production minus the rate of consumption (other factors include algal nutrition, light conditions and temperature). For example, the species of algae and their numbers in oxidation ponds are changed by the consumption of smaller algal species by zooplankton herbivores. Along the California coast, in salt evaporation ponds, Artemia consumes some species of algae, but through some form of differential digestion, allows other species to pass through the digestive tract intact.

Discussion following paper "Problems
in the Laboratory Culture of Plank-
tonic Blue-Green Algae" by W. R. Eberly

Short incubation periods, such as six hours, probably do not provide information on long-term growth rates (days or weeks) and data from such studies should not be used to extrapolate growth curves for long periods.

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It is difficult to interpret what happens to growth rates after the addition of microelements; this difficulty is compounded if the pH of the culture medium is not controlled. If the pH goes above approximately 9.0, all of the carbon in the water will be in the form of carbonate. If maximum growth occurs in cultures above a pH of 9.0, the algae may be showing a preference for carbon in the carbonate form; if true, after maximum density is reached and after the pH drops, the carbonate form will no longer be available and carbon may become a limiting factor.

Oscillatoria rubescens shows different pigmentation under different growth conditions; although little is known about the chemistry of the pigments, such knowledge may be useful in dating the past cycles of Oscillatoria in various lakes by analyzing pigments trapped in the sediments.

In major lakes in northern Minnesota, Oscillatoria agardhii and Merismopedia marssonii are found in one stratified layer, whereas Oscillatoria redecii and Merismopedia trolleri are found in another layer of the same lake. Perhaps the presence of hydrogen sulfide explains the difference by providing sulfur as a form of nutrition for O. redecii and also for M. trolleri, both of which are found in the lower stratifications where H_2S is most prevalent.

Discussion following paper "Aspects of the Nitrogen Nutrition of Naturally Occurring Populations of Phytoplankton Dominated by Blue-Green Algae" by V. A. Dugdale

In Saskatchewan, Canada, Anabaena in lakes blooms about a month after ice break-up. Following the relatively short Anabaena bloom, Aphanizomenon takes over. Nitrogen usually never shows an increase after the disappearance of the Anabaena bloom.

Under several feet of ice and snow during the winter in both Alaska and Canada, much light still penetrates into the water. Chlorophyll measurements under the ice indicate some organic production.

Discussion following paper "Environmental Requirements of Thermophilic Blue-Green Algae" by R. W. Castenholz

A hot spring at Mount Rainier has a very conspicuous mass of algae (Schizothrix calcecola) that affords refuge for herbivores. One of the herbivores, an oligochaete unique to this type of algal mass, has its own network of channels wherein it grazes. Temperature on the mat surface can be up to 55°C with temperatures under the mat at 40°C,

Summary of Floor Discussions

even though water the mat is growing in is at the higher temperature. The herbivores bore upward through the mat surface at night to graze but return to subsurface channels during the day; they probably use the surface at night because of the absence of predators.

Discussion following paper "Growth Requirements of Blue-Green Algae as Deduced from their Natural Distribution" by W. C. Vinyard

Some organisms have been observed growing in diesel and high octane fuels in which water content was less than 2%. It has been established that certain species of the fungi and bacteria are able to live in various petroleum products. Species of some algae have also been found in petroleum by-product wastes. Some blue-green algae were observed in the tar of pools in oil fields where they were existing under such darkened conditions as to make it plausible to assume that they were deriving nourishment from the hydrocarbons in the wastes. Interestingly, these algae had retained their bright blue-green color. In some waste ponds, the algae formed heavy mats intermixed with the tar and oil; the algal forms varied in color from brown to reddish, indicating that they consisted mainly of diatoms.

Water pumped up with the oil is separated and pooled in many oil fields; both brine and non-brine waters can be pumped. In non-brine water, diatoms dominate.

Discussion following paper "Recent Advances in the Physiology of Blue-Green Algae" by O. Holm-Hansen

Diamino phenolic acid is present in all blue-green algae; phenolic derivatives are also present in lichens, some bacteria, and some fungi. Diamino phenolic acid has not been found in Chlorella. Since some organisms produce special pigments and other chemical derivatives that are specific to their groups, it would enhance the field of taxonomy to utilize these characteristics as well as morphological characters. In some instances, chemical or biochemical evidence can be utilized better than morphological evidence. However, it is quite difficult at this stage of science to isolate and identify most organic compounds of various organisms, and this is probably the major reason more biochemical evidence is not used in defining taxa.

In studying productivity in laboratory cultures, pH is probably not an important factor per se, but rather, indirectly affects productivity by changing chelation of such elements as iron and manganese, permitting them to stay in solution.

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In nature, pH usually rises as algal populations increase; in areas where bloom conditions occur, the pH can be as high as 10.0 to 10.5. In the tropics, the pH fluctuates diurnally from 7 to 10.5 during 24 hours, most likely due to photosynthesis in the algae. If pH is considered an important factor in primary productivity, it should be measured continuously during the period of work, whether in the laboratory or in the field. Since there is little known about the influence of pH on growth, research should be carried out to assess the role of pH as it directly and indirectly affects growth.

GENERAL DISCUSSION OF
NUTRIENT MEASUREMENT AND NUISANCE CONTROL

September 24, 1966

Alexander M. Dollar, Michael Parker, William A. Dawson^{1/}

INTRODUCTION

Successful multiple use of lake and stream water systems entails a thorough understanding of the productivity of these systems. Lack of knowledge and understanding of the causes of algal blooms now prevents effective control. Principal offenders are blue-green algae, whose intense blooms can form floating masses of algae, which eventually die and decompose.

To initiate discussion in the morning session, participants were invited to present ideas regarding a watershed's contribution of nutrients to its lakes. A study of Upper Klamath Lake, Oregon, was chosen as a starting point for the discussion. It was not possible to record the session verbatim nor even to note the specific contributions of each person. The following is a summary of the session as a whole, often with information rearranged to make a more logical presentation than could be maintained or even anticipated in a free-flowing discussion.

MEASURING THE INFLOW OF NUTRIENTS

The morning's discussion began with a consideration of methods and measurements to evaluate a watershed's contribution of nutrients to its lakes. It was generally concluded that given adequate manpower and facilities, contributions of nutrients from the watershed can be evaluated fairly completely. Upper Klamath Lake, Oregon, (130 square miles, with a 3810 square mile watershed, and an average depth of 8 feet) was used as an example for discussion. A. F. Bartsch and other staff of the Federal Water Pollution Control Administration, in Corvallis, outlined the work they have done on this lake to measure the input from several sources:

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ENVIRONMENTAL REQUIREMENTS OF BLUE-GREEN ALGAE

1. Inflow from tributary streams. Nitrate, nitrite, ammonia, orthophosphate, iron, cobalt, molybdenum, manganese, boron, vanadium, and other elements were all analyzed in both the particulate and soluble (passing through a $0.45\ \mu$ membrane filter) fractions of samples.
2. Farm land irrigation. Some areas of lake bottom have been reclaimed from the lake by erecting low earthen dams. Return water from irrigation is collected in sumps before being returned by pumpage to the lake. Nutrients have been measured in the returning water (as for the tributaries).
3. Springs and seepage. The U. S. Geological Survey made a detailed determination of the surface water budget of the lake and by difference the volume of water contributed from springs and seepage was calculated at 17%. Analyses of the nutrients in visible springs flowing into the lake and artesian wells in the area were made, weighted, and used to estimate the amount of nutrients contributed from seepage and springs within the lake.
4. Waterfowl. The lake is on one of the major flyways, and some attempt has been made to evaluate the nitrogen and phosphorus contributed to the bottom sediments by waterfowl. It appears that less than 2% of the phosphorus entering the lake comes from this source.

METHODS

During the course of the morning several points were raised which have general pertinence in relation to specific methodology. The need for pretreatment of Millipore filters to avoid contaminating filtrates was emphasized. This is important in relation to organic matter, nitrogen, phosphorus, and some micronutrients. In nutrient-poor waters the amount of micronutrient (as Al, Si) contributed by an unwashed filter may exceed that present in the sample. The problem may not be as critical in richer waters, but should still be considered. Methods for decontaminating filters included rinsing with several hundred ml of distilled water or dilute HCl or soaking the filters in distilled water overnight.

Empirical testing should be used to determine whether the rupturing of cells during filtration or storage of samples prior to treatment introduces significant changes in nutrient concentration. As a general rule, filtration should be as gentle as possible and the time

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between taking a sample and its treatment should be minimum. A filtration vacuum of not more than one-half atmosphere has often been used.

Obtaining adequate samples to represent the whole lake both temporally and spatially is another area where preliminary investigations are needed. In most cases there will have to be some compromise between number of samples taken and time available for analysis and/or cost. In Upper Klamth Lake it was determined that three transects were adequate to describe the lake as far as the objectives of the study were concerned. Samples were taken by lowering a tube to the bottom at various points on each transect and pooling these samples for each transect. It is important to remember that the objectives of each study will probably be different and that each sampling program should be considered in relation to the problem to be solved.

Algal standing crop is often measured on the basis of cell numbers, cell volume, wet or dry weight, or chlorophyll content. Dr. Dollar suggested the use of Swinney glass filters for sampling small volumes of water containing relatively large amounts of algae. Material retained by the filters can be analyzed directly for total nitrogen and can be used for chlorophyll determinations.

The differences between much oceanographic work and that in productive fresh waters were mentioned by several discussants. Productivity in the ocean is usually less than in productive lakes, and there are also often lower concentrations of nutrients. Consequently, the methods for analysis must be more sensitive in many cases. Fluorometry is an example of a sensitive technique which has been used for the determination of chlorophyll. As used in this method, the fluorometer measures light emitted by chlorophyll molecules which have been excited by an activating wavelength of light. The method, used successfully with chlorophyll extracts, measures only chlorophyll a or total chlorophyll and cannot resolve other pigments. It has also been used to continually monitor the content of chlorophyll in a stream of untreated sea water passing through the instrument. Since the amounts of chlorophyll were near the limit of the machine's sensitivity in the latter case, special instrumentation was needed to accurately detect these responses. In fresh waters the standing crop is often much higher and this difficulty might not be such a problem. Calibration by the use of standard cultures of organisms could also aid in the method's application to rapid in situ sampling and measurement. It would seem the method has good possibility of being useful to provide at least relatively accurate in situ estimates of chlorophyll in lakes, although many technical difficulties remain to be solved.

ENVIRONMENTAL REQUIREMENTS OF BLUE-GREEN ALGAE

IMPORTANCE OF SEDIMENTS AS A SOURCE OF NUTRIENTS

The study of Upper Klamath Lake also raised another question. Although it is possible to evaluate the input of nutrients to a lake, the question arises as to the relative importance of allochthonous nutrients as opposed to those regenerated from sediments. This question is of acute importance for those charged with the responsibility of predicting changes in lakes resulting from changes in the composition of the nutrients in the incoming waters. In Upper Klamath Lake it was calculated that the upper one inch of sediment has an amount of nutrients equivalent to that brought into the lake by approximately 60 years of allochthonous supply. However, there seems to be a lack of data which can be used to quantitatively evaluate the contribution of nutrients which are recycled from the sediments.

If, in any given case, it is decided that the contribution of nutrients from the sediments has a decisive influence on the algal population, can anything be done to eliminate this contribution? One possibility which has already been investigated involved placing about six inches of nutrient-poor soil over rich marsh land. This effectively sealed off the nutrients in the marsh from regeneration and release into the water after the basin was filled.

Similar approaches might also be used in lakes which are already formed. However, the intended purpose of any such treatment would need to be carefully evaluated. Any procedure which completely eliminated the contribution of nutrients from the sediments would also of necessity eliminate much or all of the benthic fauna living there. This, in turn, would have an effect on other organisms, as fish, which are in some way dependent on benthos. Thus, if such methods are to be utilized as a management tool, careful consideration of the management's objectives will need to be made. Complete elimination of nutrient regeneration from sediments might lead to more aesthetic conditions as far as algal nuisance blooms are concerned, but at the same time might severely hamper recreational activities such as fishing.

CONTROL OF ALGAL BLOOMS

The problem of controlling algal blooms was raised several times, and there was some discussion of what can be done to alleviate their development. One proposal, already discussed, was to seal off the bottom of the lake, or a significant proportion of it, to reduce the availability of recycled nutrients. This approach would seem to be most practical in the case of smaller impoundments which have yet to be filled with water and much less so in larger lakes already existing.

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Secondly, although the precise importance to algal blooms of the release of nutrients from sediments is uncertain, it is known from experience that decreasing or changing the nutrient composition of the incoming water may lead to changes in the algal flora. Thus, in the Madison, Wisconsin, area, after sewage was diverted from several lakes, surface blooms of algae were greatly reduced. In Lake Waubesa the amount of algae, in mg/liter, remained the same as before diversion, but instead of a community composed largely of one or two species which formed blooms, there are now several scores of species, none of which predominates to form the previous nuisance.

Several similar situations are under study in the State of Washington. The city of Seattle has almost finished completely diverting sewage from Lake Washington, and studies are in progress to follow the changes which will occur as the lake is flushed. The main source of incoming water is quite low in nutrients.

In Green Lake an experiment is under way in which the lake is being flushed by large volumes of the city of Seattle's water. The total phosphate concentration has been reduced to one-half its normal value at the beginning of the summer but returns to "normal" levels later in the summer. Although data available are inconclusive, there is some indication that blooms early in the year have been reduced even though conditions toward the end of summer appear unchanged.

It was felt that in many cases sealing off a lake's bottom or reducing nutrient concentrations by flushing would lead to changes in the relative proportions and/or types of algae present in the system. Dr. Prowse, Dr. Edmondson, and others felt that a possible factor contributing to this change, or reinforcing it, would be changes in the zooplankton and in their grazing patterns. This effect of selective feeding by zooplankton necessitates further studies on their distribution and abundance and their role in controlling algal populations.